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# Released granulocytic elastase: An indicator of pathobiochemical alterations in septicemia after abdominal surgery

Karl-Heimo Duswald, M.D., Marianne Jochum, Ph.D., Wolfgang Schramm, M.D., and Hans Fritz, Ph.D., Munich, West Germany

*To discover the role of lysosomal enzyme release from polymorphonuclear (PMN) leukocytes during septicemia, plasma levels of PMN elastase were measured with a newly developed enzyme-linked immunosorbent assay for detection of the PMN elastase- $\alpha_1$ -proteinase inhibitor complex (E- $\alpha_1$ PI). Plasma samples from 41 patients were assayed continuously before and after major abdominal surgery. The patients were divided into a group without infection (group A) and two septicemia groups (survivors in group B and nonsurvivors in group C). The E- $\alpha_1$ PI levels of the 11 patients in group A without any signs of pre- or postoperative infection were in the normal range (a normal value of  $86.5 \pm 25.5$  ng/ml has been reported in 153 healthy subjects), except for a small increase to  $208.8 \pm 25.6$  ng/ml 12 hours after surgery. When septicemia was confirmed clinically in patients in groups B and C, the E- $\alpha_1$ PI levels rose on average to six times the norm in group B ( $649.9 \pm 116.3$  ng/ml) and to more than 10 times the norm in group C ( $985.0 \pm 154.6$  ng/ml). Peak values  $>2,200$  ng/ml could be measured in both groups. In patients in group B, the E- $\alpha_1$ PI levels returned to normal during recovery, while in those in group C they remained significantly elevated ( $560.5 \pm 174.7$  ng/ml) until death. Correlations were demonstrated between the amount of elastase released into the circulation and the decrease in the activities of antithrombin III, coagulation factor XIII, and  $\alpha_2$ -macroglobulin, as well as the increased C-reactive protein in plasma. We conclude that release of elastase and other lysosomal factors from PMN cells plays a major role in the pathobiochemical alterations during septicemia. In addition, significantly elevated E- $\alpha_1$ PI levels in the postoperative course seem to be a suitable indicator for onset and persistence of sepsis as well as of the severity of this disorder in patients after major surgery.*

*From the Surgical Clinic, Department of Clinical Chemistry and Biochemistry, and Medical Clinic, University of Munich, Munich, West Germany*

DESPITE RECENT PROGRESS in prophylaxis and therapy of postoperative sepsis, the high mortality rate is still a major problem (30% to 70% after septicemia, >70% after septic shock).<sup>20-22</sup> The underlying pathomechanisms of multiple organs failure due to septicemia are not sufficiently understood. Lysosomal proteinases as well as hydrogen peroxide or oxygen radicals released

from stimulated or disintegrating polymorphonuclear (PMN) leukocytes enhance the inflammatory response by destruction of connective tissue structures and cell membrane constituents<sup>11, 33</sup> as well as plasma proteins either by proteolytic degradation or denaturation by oxidation. There may be a release of toxic peptides.<sup>2, 6, 7, 17, 29-32</sup>

In this study we used PMN elastase as a marker of such pathologic release reactions. The extracellularly liberated elastase competes with susceptible protein substrates including  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ PI) and  $\alpha_2$ -macroglobulin ( $\alpha_2$ M), being finally eliminated as inactive enzyme-inhibitor complexes (90% as  $\alpha_1$ PI complex and 10% as  $\alpha_2$ M complex) via the reticuloendothelial system.<sup>25, 27</sup> Therefore, the PMN elastase-

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Reprint requests: Karl-Heimo Duswald, M.D., Chirurgische Klinik Innenstadt der Universität München, Nußbaumstrasse 20, D-8000 München 2, West Germany.

**Table I.** Clinical data of the three patient groups

| Patient No.                    | Age (yr) | Sex | Malignancy | Operation             | Source of sepsis | Onset of sepsis (days after surgery) | Outcome* |
|--------------------------------|----------|-----|------------|-----------------------|------------------|--------------------------------------|----------|
| Group A (without infection)    |          |     |            |                       |                  |                                      |          |
| 1                              | 45       | M   | +          | Rectum resection      |                  |                                      | 15       |
| 2                              | 40       | M   | -          | Choledochojejunostomy |                  |                                      | 10       |
| 3                              | 48       | M   | +          | Gastrectomy           |                  |                                      | 22       |
| 4                              | 67       | F   | -          | Colon resection       |                  |                                      | 12       |
| 5                              | 62       | F   | -          | Colon resection       |                  |                                      | 19       |
| 6                              | 29       | M   | +          | Whipple procedure     |                  |                                      | 13       |
| 7                              | 51       | M   | -          | Stomach resection     |                  |                                      | 8        |
| 8                              | 69       | F   | +          | Colon resection       |                  |                                      | 11       |
| 9                              | 63       | F   | +          | Colon resection       |                  |                                      | 10       |
| 10                             | 31       | F   | +          | Papillotomy           |                  |                                      | 9        |
| 11                             | 24       | M   | -          | Colon resection       |                  |                                      | 16       |
| Group B (sepsis with recovery) |          |     |            |                       |                  |                                      |          |
| 1                              | 69       | F   | -          | Colon resection       | Peritonitis      | 1                                    | 11       |
| 2                              | 20       | M   | -          | Portocaval shunt      | Pneumonia        | 2                                    | 12       |
| 3                              | 50       | M   | -          | Stomach resection     | Peritonitis      | 6                                    | 9        |
| 4                              | 49       | F   | +          | Gastrectomy           | Peritonitis      | 8                                    | 19       |
| 5                              | 62       | M   | -          | Liver resection       | IA               | 1                                    | 10       |
| 6                              | 40       | F   | -          | Papillotomy           | IA               | 1                                    | 12       |
| 7                              | 70       | M   | +          | Pancreas resection    | IA               | 1                                    | 22       |
| 8                              | 79       | F   | -          | Papillotomy           | Pneumonia        | 4                                    | 8        |
| 9                              | 30       | M   | -          | Replantation of thigh | Wound abscess    | 3                                    | 13       |
| 10                             | 52       | M   | -          | Pancreas resection    | Peritonitis      | 2                                    | 14       |
| 11                             | 45       | M   | -          | Jejunum resection     | Peritonitis      | 1                                    | 4        |
| 12                             | 65       | M   | +          | Jejunum resection     | IA               | 1                                    | 14       |
| 13                             | 43       | M   | -          | Pancreas resection    | IA               | 2                                    | 18       |
| 14                             | 77       | F   | +          | Ileum resection       | IA               | 1                                    | 6        |
| Group C (sepsis with death)    |          |     |            |                       |                  |                                      |          |
| 1                              | 58       | M   | +          | Pancreas resection    | Peritonitis      | 7                                    | 9        |
| 2                              | 54       | M   | -          | Stomach resection     | Peritonitis      | 6                                    | 81       |
| 3                              | 40       | F   | -          | Stomach resection     | Peritonitis      | 22                                   | 12       |
| 4                              | 67       | M   | +          | Rectum extirpation    | Peritonitis      | 6                                    | 57       |
| 5                              | 79       | M   | +          | Rectum extirpation    | Pneumonia        | 5                                    | 6        |
| 6                              | 60       | M   | +          | Pancreas resection    | Peritonitis      | 3                                    | 10       |
| 7                              | 51       | M   | -          | Portocaval shunt      | Pneumonia        | 7                                    | 23       |
| 8                              | 50       | F   | -          | Pancreas resection    | Peritonitis      | 41                                   | 8        |
| 9                              | 68       | M   | +          | Colon resection       | Wound abscess    | 27                                   | 23       |
| 10                             | 46       | M   | -          | Stomach resection     | Peritonitis      | 11                                   | 30       |
| 11                             | 69       | F   | +          | Ileum resection       | Peritonitis      | 1                                    | 6        |
| 12                             | 68       | M   | -          | Pancreas resection    | Peritonitis      | 1                                    | 32       |
| 13                             | 60       | M   | +          | Gastrectomy           | Peritonitis      | 5                                    | 12       |
| 14                             | 63       | M   | -          | Jejunum resection     | Peritonitis      | 1                                    | 11       |
| 15                             | 73       | F   | -          | Stomach resection     | Peritonitis      | 1                                    | 9        |
| 16                             | 78       | F   | -          | Colon resection       | Peritonitis      | 1                                    | 1        |

Legend: M = male; F = female; IA = intra-abdominal abscess.

\*Group A, discharge (days after surgery); group B, recovery (days after onset); group C, death (days after onset).

$\alpha_1$ PI (E- $\alpha_1$ PI) complex should be a suitable indicator for lysosomal enzyme release. The aims of our study were threefold: (1) to show the influence of major surgery on PMN elastase release; (2) to verify the

effect of postoperative infections on E- $\alpha_1$ PI levels; and (3) to investigate a possible correlation between E- $\alpha_1$ PI levels, patient outcome, and the consumption of important blood proteins.

## MATERIAL AND METHODS

**Patients.** From March 1980 to June 1981, 41 patients were investigated according to the study protocol before major abdominal surgery and during the postoperative course. Patients were attributed to one of three clearly defined groups. In group A, the 11 patients showed no signs of infection pre- and postoperatively. In group B, the 14 patients had septicemia in the postoperative course but survived. In group C, the 16 patients died due to septicemia after major abdominal surgery. Clinical data of the three patient groups are listed in Table I. The diagnosis of septicemia was established by: (1) clearly defined source(s) of sepsis with positive culture of the infectious organisms; (2) leukocyte counts  $>15,000/\mu\text{l}$  or  $<5000/\mu\text{l}$ ; (3) platelet counts  $<100,000/\mu\text{l}$  or a platelet drop  $>30\%$  within 24 hours. Positive blood culture was not presupposed but was registered in 30% of patients in group B and in 50% of patients in group C. Septic shock (defined by cardiac index  $>6$  L/min/m<sup>2</sup> and systemic vascular resistance  $<600$  dynes  $\cdot$  sec/cm<sup>5</sup>) could be demonstrated in nine patients in group C but in no patient in group B.

**Sampling procedure.** Blood sampling and registration of all clinical parameters were performed 12 hours before surgery, 12 hours after surgery, and every 12 hours thereafter until discharge (group A), recovery from any sign of infection (group B), or death due to septicemia (group C).

Apart from all laboratory data normally monitored for seriously ill patients, plasma samples for detection of specific parameters such as E- $\alpha_1$ PI, antithrombin III (AT III), coagulation factor XIII (F XIII),  $\alpha_2$ M, and C-reactive protein (CRP) were obtained by drawing 4.5 ml of venous blood into plastic syringes containing 0.5 ml of sodium citrate (2.2 gm per 100 ml distilled water). Plasma was separated from blood cells within 30 minutes after sample collection to prevent leakage of leukocyte constituents. Plasma samples were stored at  $-70^\circ\text{C}$  until assayed. Plasma levels of patients receiving banked blood with high levels of complexed elastase<sup>12,14</sup> were corrected for the transfused amount, taking into account the elimination rate of the complex in vivo (half-life equals 1 hour).

**Bacteriologic data.** Microbiologic examinations were performed in wound secretions, abdominal drainages, tracheobronchial secretions, closed drainage systems of indwelling urethral catheters, and blood. Specimens were collected every 2 days. Additional indications for performing blood cultures were a sudden increase in the patient's temperature ( $>38.5^\circ\text{C}$ ), a change in sensorium, and the onset of chills. In these

cases, samples were taken at least three times within a 24-hour period. Other secretions were collected by established techniques. Results of microbiologic cultures and antimicrobial susceptibility tests were tabulated by 2 days after collection.

**Hematologic data.** Leukocytes were counted by an electronic counter (Coulter Counter, model B, Coulter Electronics Inc., Hialeah, Fla.) and thrombocyte counts were performed with the Neubauer counting chamber (Assistant, Sontheim, West Germany).

### Plasma factors

**Enzyme-linked immunosorbent assay for E- $\alpha_1$ PI.** With the technique of Neumann et al.,<sup>23,24</sup> E- $\alpha_1$ PI was determined by a two-site sandwich enzyme-linked immunosorbent assay including antisera against both elastase and  $\alpha_1$ PI (E. Merck, Darmstadt, West Germany). Concentrations (in nanograms per milliliter) are given for the amount of complexed elastase only.

**AT III, F XIII,  $\alpha_2$ M, and CRP.** The inhibitory activity of AT III against thrombin was determined with use of the chromogenic peptide substrate S-2238 (Deutsche Kabi, Munich, West Germany). The biologic activity of the fibrin-stabilizing factor F XIII was measured by a commercial test system (Factor XIII-Schnelltest; Behring Werke AG, Marburg, West Germany). Both assays were performed as previously described.<sup>16</sup> Plasma levels of F XIII subunits A and S were detected according to Laurell<sup>19</sup> with the use of monospecific antisera from Behring Werke AG (Clotimmun-Faktor XIII-A, Clotimmun-Faktor XIII-S). The inhibitory activity of  $\alpha_2$ M was determined with a commercial test system ( $\alpha_2$ -Macroglobulin Test Combination; Boehringer, Mannheim, West Germany) according to Ganroth.<sup>9</sup> Plasma concentrations of CRP and  $\alpha_2$ M were evaluated by a radial immunodiffusion technique with standardized immunodiffusion plates (LC Partigen CRP, M-Partigen  $\alpha_2$ -Makroglobulin; Behring Werke AG).

**Classification of data.** To allow comparison between measured data, mean values are presented for identical clinical periods: period 1, 12 hours before operation; period 2, 12 hours after operation; period 3, postoperative course without infection (group A) or before onset of sepsis (groups B and C); period 4, time of onset of sepsis (groups B and C); period 5, course after onset of sepsis (groups B and C); and period 6, day of discharge (group A), day of recovery from infection (group B), or day of death due to septicemia (group C).

**Statistics.** Unless otherwise stated, results are given as the percentage of the value obtained in a standard

**Table II.** Mean values ( $\pm$  SEM) E- $\alpha_1$ PI (ng/ml)

| Group                 | Period 1<br>(12 hr before<br>operation) | Period 2<br>(12 hr after<br>operation) | Period 3<br>(postoperative<br>course) | Period 4<br>(onset<br>of sepsis) | Period 5<br>(course<br>of sepsis) | Period 6<br>(discharge, recovery,<br>or death) |
|-----------------------|---|--|---------------------------------------|----------------------------------|-----------------------------------|--|
| A                     | 90.9 $\pm$ 7.6                          | 208.8 $\pm$ 25.6                       | 143.8 $\pm$ 10.8                      |                                  |                                   | 96.0 $\pm$ 5.6                                 |
| B                     | 99.2 $\pm$ 18.6                         | 258.9 $\pm$ 25.9                       | 316.4 $\pm$ 25.0                      | 646.9 $\pm$ 116.3                | 266.1 $\pm$ 35.4                  | 93.2 $\pm$ 7.6                                 |
| C                     | 353.4 $\pm$ 71.5                        | 347.3 $\pm$ 46.6                       | 229.1 $\pm$ 15.9                      | 985.0 $\pm$ 154.6                | 517.6 $\pm$ 41.6                  | 560.5 $\pm$ 174.7                              |
| Student <i>t</i> test |   |  |                                       |                                  |                                   |  |
| A:B                   | NS                                      | NS                                     | $p < 0.0005$                          |                                  |                                   | NS   |
| A:C                   | $p < 0.025$                             | $p < 0.01$                             | $p < 0.0005$                          |                                  |                                   | $p < 0.0125$                                   |
| B:C                   | $p < 0.025$                             | NS                                     | $p < 0.0125$                          | NS                               | $p < 0.0005$                      | $p < 0.0125$                                   |

**Table III.** Mean values ( $\pm$  SEM) of AT III (% of normal activity), F XIII (% of normal activity), and  $\alpha_2$ M (% of preoperative inhibitory activity) in patients in groups A, B, and C

|              | Period 1<br>(12 hr before<br>operation) | Period 2<br>(12 hr after<br>operation) | Period 3<br>(postoperative course) | Period 4<br>(onset of sepsis) | Period 5<br>(course of sepsis) | Period 6<br>(discharge, recovery,<br>or death) |
|--------------|---|--|------------------------------------|-------------------------------|--------------------------------|--|
| Group A      |   |  |                                    |                               |                                |  |
| AT III       | 97.1 $\pm$ 7.6                          | 83.8 $\pm$ 5.4                         | 83.8 $\pm$ 1.9                     |                               |                                | 103.8 $\pm$ 6.7                                |
| F XIII       | 71.9 $\pm$ 3.1                          | 43.7 $\pm$ 6.2                         | 44.4 $\pm$ 1.2                     |                               |                                | 65.6 $\pm$ 10.7                                |
| $\alpha_2$ M | 100                                     | 87.5 $\pm$ 3.6                         | 87.7 $\pm$ 1.8                     |                               |                                | 97.3 $\pm$ 4.2                                 |
| Group B      |   |  |                                    |                               |                                |  |
| AT III       | 84.3 $\pm$ 10.2                         | 64.9 $\pm$ 9.2                         | 53.6 $\pm$ 9.4                     | 50.4 $\pm$ 4.4                | 62.4 $\pm$ 2.1                 | 93.0 $\pm$ 8.3                                 |
| F XIII       | 68.0 $\pm$ 6.5                          | 53.2 $\pm$ 5.9                         | 45.0 $\pm$ 3.8                     | 37.5 $\pm$ 8.8                | 40.6 $\pm$ 3.1                 | 88.6 $\pm$ 8.1                                 |
| $\alpha_2$ M | 100                                     | 63.5 $\pm$ 6.2                         | 61.2 $\pm$ 6.6                     | 50.0 $\pm$ 4.9                | 57.8 $\pm$ 1.5                 | 75.4 $\pm$ 5.5                                 |
| Group C      |   |  |                                    |                               |                                |  |
| AT III       | 81.1 $\pm$ 14.3                         | 77.6 $\pm$ 7.6                         | 56.7 $\pm$ 3.8                     | 50.4 $\pm$ 5.1                | 52.1 $\pm$ 2.5                 | 45.8 $\pm$ 6.2                                 |
| F XIII       | 70.0 $\pm$ 13.3                         | 62.5 $\pm$ 7.2                         | 49.7 $\pm$ 2.0                     | 46.8 $\pm$ 5.9                | 29.9 $\pm$ 4.3                 | 28.1 $\pm$ 10.6                                |
| $\alpha_2$ M | 100                                     | 77.3 $\pm$ 2.6                         | 72.6 $\pm$ 1.3                     | 62.2 $\pm$ 4.5                | 69.8 $\pm$ 1.5                 | 72.2 $\pm$ 6.2                                 |

plasma pool (mean  $\pm$  SEM). Statistical evaluation was performed by the Student *t* test;  $p$  values  $\leq 0.05$  were considered significant.

## RESULTS

### Septic parameters

*Primary source of septicemia and bacteriologic results.* Clinical data are summarized in Table I. Peritonitis was the primary source of septicemia in patients in groups B and C, followed by pneumonia and wound abscess. Bacteriologic data from a septical focus showed mainly mixed cultures. In patients in group C, gram-negative bacteria and fungal infections predominated (15 patients in group C and eight in group B). More than  $10^4$  colony forming units of *Candida albicans* were found in three patients in group B and in 12 in group C. Gram-positive cocci were equally distributed (10 and 11 patients in groups B and C, respectively). Anaerobic bacteria were detected in five patients in group B and only one patient in group C. Blood cultures were found to be positive during

onset of sepsis in patients 1, 5, and 8 in group B and in patients 1, 3, 5, 6, 10, 11, 15, and 16 in group C.

*Temperature, leukocyte count, and thrombocyte count.* Patients in group A showed a slight rise in temperature ( $<38^\circ$  C) after surgery but no significant changes in leukocyte or thrombocyte counts compared with normal values. During infection in patients in groups B and C we recorded temperatures  $>38^\circ$  C, leukocyte counts  $>12,000/\mu\text{l}$  or  $<5000/\mu\text{l}$ , and platelet counts  $<125,000/\mu\text{l}$ . Four patients in group B (Nos. 1, 3, 9, and 12) and four in group C (nos. 1, 8, 13, and 15) showed leukopenia  $<5000$  cells/ $\mu\text{l}$  during the onset of sepsis. In 10 of 14 patients in group B and in 11 of 16 patients in group C an absolute level  $<100,000$  thrombocytes/ $\mu\text{l}$  could be measured during onset of sepsis. In the other patients in both groups the thrombocyte count was diminished  $>30\%$  compared with the level 24 hours earlier.

### Plasma factors

*E- $\alpha_1$ PI.* The mean ( $\pm$ SEM) values in each group for the different periods are listed in Table II. In

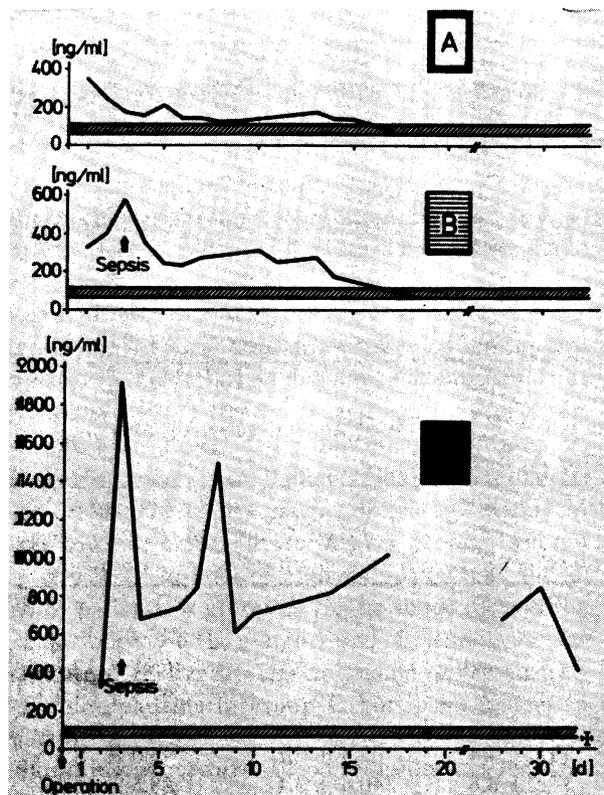


Fig. 1. Typical individual graphs of E- $\alpha_1$ PI during the postoperative course of patients in groups A (without infection; patient no. 3), B (sepsis with recovery; patient no. 7), and C (sepsis with death; patient no. 12).

patients without any sign of preoperative infection (groups A and B), the operative trauma was followed by an increase of E- $\alpha_1$ PI levels up to threefold the norm ( $86.5 \pm 25.5$  ng/ml). Group C showed significantly elevated E- $\alpha_1$ PI levels before operation compared with the norm; a slight but not significant decrease could be observed 12 hours after operation. This is because six of 16 patients of these group were operated on because of peritonitis. They had higher leukocyte counts ( $11,600 \pm 2600$  cells/ $\mu$ l, i.e., significantly more than in groups A and B) but showed no other sign of septicemia.

In the later postoperative phase there was clear normalization in group A, whereas the E- $\alpha_1$ PI levels in groups B and C showed a moderate but not significant elevation compared with the levels 12 hours after operation. At the onset of septicemia, however, a highly significant increase in the E- $\alpha_1$ PI levels could be detected, up to sixfold in group B and up to tenfold in group C. Peak levels were  $>2200$  ng/ml in both groups. The E- $\alpha_1$ PI levels of patients with sepsis who

recovered showed a clear tendency toward normalization. When septicemia persisted, however, levels of E- $\alpha_1$ PI were high until death.

Fig. 1 demonstrates typical individual graphs of the E- $\alpha_1$ PI concentration for one patient in each group. While group A showed a moderate elevation of E- $\alpha_1$ PI levels in the early phase after surgery only, in group B higher peak levels as well as a prolonged period of elevated E- $\alpha_1$ PI levels was observed. Group C showed very high levels several times and no tendency towards normalization. At the time of death the levels were still high.

*AT III, F XIII, and  $\alpha_2$ M.* Concomitant with the increase in E- $\alpha_1$ PI there was a significant decrease of AT III, F XIII, and  $\alpha_2$ M (Table III). These proteins or inhibitors are known to be easily susceptible to proteolytic degradation or cleavage ( $\alpha_2$ M) by elastase or other lysosomal proteinases. The diminished activities at onset of septicemia were normalized in all patients overcoming the infection, whereas a further significant decrease was found in group C (lethal outcome).

Values for  $\alpha_2$ M are given as the percentage of preoperative levels, because they were already low before surgery. Thereby, remarkable differences were found between the patients with and without sepsis. While the highest loss of inhibitory activity was only 13% in group A, it reached 50% in group B and 40% in group C. At the time of the last measurement, group A showed normal values, whereas those in groups B and C were still significantly decreased. Remarkably, both the inhibitory activity of  $\alpha_2$ M and the immunologically measured concentration were likewise diminished. Forty-three pairs of values of both measurements were selected for linear regression analysis. The correlation coefficient ( $r = 0.9155$ ) confirmed that there was no difference between concentration and inhibitory activity of  $\alpha_2$ M within the three groups after major surgery.

*CRP.* Preoperative values were found to be close to normal (0.05 to 1.0 mg/100 ml<sup>18</sup>) in groups A and B but were elevated in group C (group A,  $1.8 \pm 0.9$  mg/100 ml; group B,  $2.8 \pm 1.7$  mg/100 ml; group C,  $4.5 \pm 2.1$  mg/100 ml). During the postoperative course, CRP plasma levels could be differentiated between patients without infection (group A,  $6.1 \pm 0.7$  mg/100 ml) and those showing signs of beginning septicemia (group B,  $14.9 \pm 1.6$  mg/100 ml; group C,  $15.1 \pm 1.5$  mg/100 ml). During sepsis onset, both groups reached equally high CRP levels (group B,  $15.1 \pm 1.4$  mg/100 ml; group C,  $15.1 \pm 2.6$  mg/100 ml). During sepsis there was a decrease in CRP in both

groups, but this did not reflect patient outcome (group B,  $9.7 \pm 0.7$  mg/100 ml; group C,  $9.9 \pm 0.7$  mg/100 ml). Only at the time of the last measurement were values in groups A and B again normal, but those of group C were further elevated (group A,  $2.4 \pm 0.6$  mg/100 ml; group B,  $2.5 \pm 0.6$  mg/100 ml; group C,  $8.8 \pm 1.4$  mg/100 ml).

## DISCUSSION

Detailed analyses of various components of the clotting, fibrinolysis, complement, and kallikrein-kinin systems performed in clinical as well as experimental studies indicate continuous activation of these cascade systems during septicemia, septic shock, or endotoxemia.<sup>1-5, 8, 15, 16</sup> Leukocytes, especially the PMN cells, are supposed to play a central role in such pathobiochemical events.<sup>6, 17, 32</sup> Recently, Aasen et al.<sup>2</sup> observed in an experimental study (lethal endotoxin shock in dogs) a relationship between the initial drop in leukocyte levels (probably combined with degranulation of these cells) and the appearance of E- $\alpha_1$ PI in plasma. They attributed the disturbances or activation of the cascade systems mainly to the action of liberated leukocytic proteinases.

So far, only Egbring et al.<sup>5</sup> have tried to measure E- $\alpha_1$ PI levels in patients with sepsis by rocket immunoelectrophoresis.<sup>19</sup> However, levels of complexed elastase as low as 0.1  $\mu$ g/ml plasma cannot be quantitated by this method. Moreover, from this study no statement is available concerning the course of E- $\alpha_1$ PI levels during septicemia. With the newly developed enzyme-linked immunosorbent assay, the detection limit of E- $\alpha_1$ PI is 0.2 ng per assay, i.e., 20 ng per milliliter of plasma. Therefore, E- $\alpha_1$ PI levels in plasma samples of healthy individuals (mean value  $86.5 \pm 25.5$  ng/ml) could be accurately measured.<sup>23</sup> The presence of the complex in normal plasma confirms the assumption of a continuous release of low doses of lysosomal PMN proteases into the circulation.

Our data are comparable with those of Ohlsson and Olsson,<sup>26</sup> who found 135 ng of granulocytic elastase per milliliter of freshly drawn normal human serum applying a specific radioimmunoassay. With a radioimmunoassay developed by Plow and Plescia,<sup>28</sup> elastase-related antigen was assessed to be 24 ng/ml in five normal plasma samples. The corresponding serum specimens showed a mean value of 326 ng/ml. Because we observed similar irregular rises in the E- $\alpha_1$ PI level during serum formation, plasma samples should be used exclusively in clinical studies.

To our knowledge, our study represents the first and most extensive documentation of granulocytic elastase

release during septicemia after major surgery. In patients without preoperative infections, surgical trauma was followed by a moderate liberation of elastase, most likely as a result of enhanced phagocytotic activity of leukocytes. The a priori elevated E- $\alpha_1$ PI level in patients who already have preoperative infections showed no further increases after surgery. Thus the release of elastase induced by surgical trauma might have been compensated for by a reduced leukocyte response due to elimination of the infectious focus.

In the postoperative phase, the mean E- $\alpha_1$ PI concentrations correlated well with the severity of infection. Highly elevated E- $\alpha_1$ PI levels at sepsis onset were normalized in those patients recovering from septicemia, whereas in the fatal cases a continuous increase of the complex levels was measured. Possibly, the high endotoxin levels (determined by a modified Limulus test)\* in the plasma specimens of some of the latter patients were responsible for this enhanced elastase liberation.

Parallel to the increase in E- $\alpha_1$ PI levels there was a decrease in levels of F XIII subunit S (carrier protein) in groups B and C, although the absolute degree of carrier protein consumption did not correspond in each case to the amount of E- $\alpha_1$ PI. On the other hand, patients without infection (group A) and normal or only slightly elevated E- $\alpha_1$ PI levels had F XIII subunit S concentrations in the normal range but often clearly reduced fibrin-stabilizing activity (due to F XIII subunit A). As demonstrated by Egbring et al.<sup>5</sup> and Ikematsu et al.,<sup>10</sup> reduction of both subunits of F XIII cannot be due to activation of the clotting cascade alone. During clotting, i.e., by the activation of thrombin, subunit A is consumed simultaneous with the F XIII activity, but subunit S is not. These data and our results suggest that in patients suffering from septicemia, unspecific proteolytic degradation by granulocytic elastase or other lysosomal proteinases is involved to a significant degree in the depletion of F XIII.

Moreover, the very low AT III activity in patients with permanently elevated E- $\alpha_1$ PI levels may also be due, at least in part, to degradation by elastase. This conclusion is based on recent in vitro studies showing that purified AT III is rapidly inactivated by isolated granulocytic elastase.<sup>13</sup>

$\alpha_2$ M, probably the most important proteinase inhibitor of the organism, is responsible for the inhibition and elimination of nearly all types of neutral and acid proteinases liberated from various body cells under physiologic and pathologic conditions. The half-life of

\*Stemberger A: Personal communication.

$\alpha_2$ M-proteinase complexes in the circulation is believed to be 10 to 12 minutes.<sup>25</sup> In our study no difference between activity and concentration of  $\alpha_2$ M could be observed. From this fact we conclude that the clearance function of the reticuloendothelial system was not impaired in our patients, although high amounts of lysosomal proteinases were liberated.

In contrast to the consumption of diverse plasma proteins, a marked rise of the acute-phase reactants occurs during infection. The acute-phase reaction, in which proteins of different function and origin participate, is thought to represent a systemic host response to injury.<sup>34</sup> Of all known factors, CRP reacts most rapidly and significantly. Because of its various biologic functions (e.g., recognition of microorganisms; activation of the classic complement pathway; reaction with lymphoid cells, phagocytic cells, and platelets<sup>18</sup>, CRP seems to play a central role in unspecific host defense. Therefore, comparison of the acute-phase response of CRP with the degree of liberated granulocytic elastase was of special interest to us. Preoperative and postoperative values of CRP and E- $\alpha_1$ PI in the control and septicemia groups designed both factors as similarly sensible to the inflammatory stimulus. However, in contrast to the E- $\alpha_1$ PI complex, a clear discrimination of the severity of septicemia was not indicated by the CRP level in both infected groups, either at onset of sepsis or during septicemia. Only the last determination was in agreement with clinical conditions. Obviously, the E- $\alpha_1$ PI level does reflect more specifically severe infections such as septicemia or septic shock.

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### **Elastasa granulocítica liberada después de cirugía abdominal**

Con el objeto de determinar el papel de la liberación de enzimas de lisosoma liberadas por los leucocitos polimorfonucleares (PMN) durante la septicemia, se midieron los niveles plasmáticos de PMN elastasa utilizando un método introducido recientemente de ensayo inmunosorbente con unión enzimática para la detección del complejo inhibitor de la PMN elastasa- $\alpha_1$ -proteinasa ( $E-\alpha_1PI$ ). En forma continua se examinaron muestras del plasma de 41 pacientes tomadas antes y después de cirugía abdominal mayor. Los pacientes fueron divididos en tres grupos: un grupo A no infectado, y dos grupos con septicemia: el grupo B de sobrevivientes y el grupo C de no-sobrevivientes. Los niveles de  $E-\alpha_1PI$  de los 11 pacientes sin datos de infección se encontraron dentro de límites normales (valores normales:  $86.5 \pm 25.5$  ng/ml determinados en 153 voluntarios sanos) con excepción de un pequeño aumento a  $208.8 \pm 25.6$  ng/ml a las doce horas del postoperatorio. Cuando se confirmó la presencia de septicemia en los pacientes de los grupos B y C, los niveles de  $E-\alpha_1PI$  se elevaron un promedio de 6 veces sobre los valores normales en los pacientes B ( $649.9 \pm 116.3$  ng/ml) y 10 veces en los pacientes del grupo C ( $985.1 \pm 154.6$  ng/ml). En ambos grupos se llegaron a medir valores máximos de 2200 ng/ml. En los pacientes del grupo B los valores de  $E-\alpha_1PI$  regresaron a lo normal durante del periodo de recuperación, en tanto que en los pacientes del grupo C, permanecieron elevados hasta el momento de la muerte ( $560.5 \pm 174.7$  ng/ml). Se demostró correlación entre la cantidad de elastasa liberada en la circulación y la disminución de la actividad de antitrombina III (AT III), factor de coagulación XIII (F XIII), y macroglobulina- $\alpha_2$  ( $\alpha_2M$ ), así como con el aumento de proteína C reactiva del plasma (CRP). Concluimos de este estudio que la liberación de elastasa y otros factores del lisosoma de las células PMN, juegan un papel muy importante en las alteraciones pato-bioquímicas que ocurren durante la septicemia. Además, consideramos que la presencia de niveles elevados de  $E-\alpha_1PI$  en el postoperatorio parece ser un indicador de la aparición y persistencia de infección, así como de la severidad del problema.

# Index to volume 98

## AUTHOR INDEX\*

### A

Abraham VS, 579  
Adams RB, 72  
Albert A, 500  
Alden P, 396  
Alexander F, 207  
Alford BA, 121  
Almqvist PM, 344  
Alonso DR, 465  
Altergott R, 1121  
Anderberg B, 1141  
Andersen DK, 1127  
Andersen J, 81  
Araneda D, 259  
Araujo JL, 259  
Arendrup H, 81  
Armin A, 1162  
Arnaud CD, 1083  
Arnesj  B, 63  
Aronson I, 1072  
Asano K, 602  
Ascher NA, 396  
Ashley SW, 166, 174  
Auguste LJ, 784  
Aun F, 900  
Aurbach GD, 1077

### B

Bailey RW, 213  
Baker CC, 199  
Baker WH, 612  
Bakshi KR, 87  
Balch CM, 151  
Ballantyne GH, 1038  
Balshi JD, 243  
Bandyk DF, 799  
Barbato A, 1121  
Barbosa JJ, 656  
Barker CF, 251  
Barofsky I, 414  
Bartels HL, 955  
Basadonna G, 324  
Baumann FG, 547  
Becker JM, 109  
Bequemini JP, 605  
Bell GA, 20  
Belzberg H, 378  
Berg JW, 93  
Bergan JJ, 810  
Berlatzky Y, 816  
Bermes E Jr, 1031  
Berne RM, 540  
Bessey PQ, 298  
Bevins PA, 7  
Bianco RW, 396  
Bille S, 81  
Biller J, 612  
Biolini D, 900  
Bjornson HS, 816  
Block MA, 1189  
Bogard WC Jr, 283  
Boks AL, 914  
Boles ET Jr, 662  
Bonds JW Jr, 979  
Bonnet P, 949  
Booker ML, 445

Bornman PC, 1  
Bowerman R, 598  
Boyersky A, 594  
Boyd MR, 35  
Bradley EL III, 983, 1064  
Brandt SJ, 338  
Brazeau P, 700  
Brennaman BH, 126  
Brennan MD, 363  
Brennan MF, 275, 1013  
Broughan TA, 746  
Brown B, 739  
Brunicardi FC, 1127  
Bryda SL, 1064  
Bubrick MP, 689  
Buchwald H, 68, 656  
Bulkley GB, 213  
Bunt TJ, 555  
Burchett H, 700  
Byrne W, 598

### C

Cady B, 1171  
Calandra DB, 1148, 1162, 1202  
Carney JA, 363, 1054  
Cato RF, 799  
Cavarocchi NC, 525  
Cerra FB, 283, 388, 396, 632  
Chan EK, 68  
Chandler JG, 72  
Chao C-C, 942  
Cheung LY, 166, 174  
Chiou R-K, 143  
Choiniere L, 350  
Christensen CW, 35  
Christou NV, 769  
Chuang P, 25  
Chute EP, 656  
Clark OH, 1000, 1083  
Clarke-Pearson DL, 98  
Cobb LF, 324  
Cohn K, 1095  
Coleman B, 378  
Coleman RE, 98  
Colvin SB, 547  
Condon RE, 648  
Connett RJ, 625  
Connolly MM, 616  
Connolly MW, 547  
Connors M, 995  
Cooper JD, 350  
Coran AG, 598  
Corlew DS, 1064  
Corrado J, 1095  
Costerton JW, 12  
Coubret P, 605  
Crawford BG, 824  
Cronenwett JL, 472  
Cruz-Bracho MR, 465  
Cullen ML, 718, 927  
Cummings B, 752

### D

Davis RA, 845  
Davis RP, 616  
Dawson CA, 35

Debas HT, 183  
De Caterina R, 465  
de Groot GH, 914  
Deitch EA, 587  
Del Santo P, 7  
Demetriou AA, 931  
Deshpande TG, 760  
Deuel M, 492  
de Vries JE, 935  
DiGirolamo M, 1064  
Dinarello CA, 298  
Di Pietro M, 598  
Doolas A, 927  
Dowling K, 684  
Doyle PW, 20  
Drevyanko TF, 330  
Drucker WR, 625  
Duarte B, 1072  
Dubois P, 350  
Duh Q-Y, 1000, 1083  
Dunn DL, 283  
Durum SK, 199  
Duswald K-H, 892

### E

Eckhauser FE, 1101, 1113  
Eckhauser ML, 708  
Economou SG, 718, 927  
Effeny DJ, 359, 484, 492  
Egan TM, 350  
Ehrenfeld WK, 484, 492  
El-Ganzouri A, 718  
Elson MK, 143  
Ely SW, 540  
Etheredge SN, 492

### F

Fabian TC, 979  
Fabri PJ, 1045  
Fagan CJ, 423  
Fagelman KM, 594  
Farbota LM, 1148  
Farias LR, 571  
Fath JJ, 396  
Fazio VW, 861  
Feind C, 1008, 1197  
Ferguson RM, 267  
Ferrara A, 230  
Ferrell LD, 484  
Finnegan MO, 87  
Fitzpatrick LA, 1077  
Flechner SM, 54  
Fleites RA, 708  
Flinn WR, 616  
Flint LM, 131  
Flint LM Jr, 158  
Foker JE, 396  
Fonkalsrud EW, 500  
Ford WDA, 935  
Forward AD, 20  
Foshag L, 684  
Francfort JW, 243, 251  
Franco D, 949  
Fratesi G, 1038  
Freeark RJ, 506, 1121  
Freeman JB, 700  
Frey CF, 571  
Friedman CJ, 72  
Fritsch A, 1154

Fritz H, 892  
Froelich JW, 1189  
Fryd DS, 729  
Fujii T, 964  
Fujimura M, 224  
Fuller J, 484

### G

Galeotti F, 319  
Galloway JR, 405  
Gans H, 370  
Ganzel B, 516  
Gaston A, 605  
Gaudino J, 976  
Gentry LO, 25  
Gerend PL, 1000  
Gianola FJ, 414  
Gillespie P, 879  
Godwin D, 93  
Goldenberg FJ, 656  
Goldstein IR, 677  
Goldstone J, 484, 492  
Goodale RL, 68  
Gooding GAW, 1083  
G thlin J, 63  
Gott JP, 516  
Gough IR, 115  
Gower WR Jr, 1045  
Graham LM, 472  
Grange D, 949  
Greeley GH Jr, 224, 423  
Green DR, 199  
Griffith BP, 739  
Gristina AG, 12  
Grosfeld JL, 668  
Grossi EA, 547  
Guerrant RL, 72  
Gum ET, 1000  
Gunther R, 571

### H

Hall JR, 7  
Halvorsen JF, 63  
Hamburger JI, 307  
Hamburger SW, 307  
Hanumadass M, 640  
Hardesty RL, 739  
Hardy MA, 1008  
Harmel RP Jr, 662  
Harrigan C, 836  
Harris NL, 1166  
Harris S, 1083  
Hayden FG, 338  
Hechtman HB, 207  
Henderson JM, 405  
Heppe H, 1162  
Hermann RE, 746  
Hermreck AS, 824  
High R, 866  
Hitchcock CR, 656  
Hobgood CD, 12  
Holcroft JW, 571  
Homburger HA, 525  
Horn SD, 213  
Hortobagyi GN, 792  
Howard RJ, 126  
Hulton NR, 291  
Hunter CE, 547  
Hyland BJ, 388

\*July, pp. 1-130; August, pp. 131-370; September, pp. 371-618; October, pp. 619-860; November, pp. 861-988; December, pp. 989-1222.

## I

Ikeda S, 313  
Iliopoulos JI, 824  
Ilstrup DM, 1054  
Imbembo AL, 708  
Itoh K, 151  
Ivankovich AD, 718  
Izquierdo R, 1031

## J

Jackson CE, 1189  
Jacobs DM, 689  
Jacobsen F, 81  
Jaffe BM, 230  
Jagelman DG, 861  
Jeckel J, 914  
Jeevanandam M, 275  
Jensen CH, 81  
Jochum M, 892  
John E, 1072  
Johnson DJ, 291  
Johnston MR, 35  
Jonasson O, 1072  
Jones CW, 30  
Jones RS, 907  
Jörtsö E, 1141  
Joseph SA, 344

## K

Kahan BD, 54  
Kagan RJ, 640  
Kambouris AA, 1189  
Kaminski DL, 760  
Kaplan A, 532  
Kaplinksky E, 532  
Katz I, 777  
Katzmark S, 516  
Kaufman DS, 1166  
Kawauchi M, 602  
Kazarian KK, 7  
Keller GA, 388  
Kempczinski RF, 816  
Kestenberg A, 109  
Khalil T, 423  
Kimose HH, 81  
King DR, 662  
Knigge KM, 344  
Kobzik L, 207  
Koch MO, 561  
Koike Y, 964  
Konomi K, 45  
Konstantinides FN, 396, 632  
Konstantinides NN, 632  
Kooy PPM, 914  
Kortz WJ, 907  
Krieger KH, 547  
Krupski WC, 359, 492  
Kuenzig M, 344  
Kupiec-Weglinski JW, 259  
Kupper TS, 199  
Kutner MH, 405

## L

Lakeman AD, 338  
LaMorte WW, 445  
Lange PH, 143  
Langer B, 752  
Langer JC, 752  
Larson GM, 236  
Laufer H, 784  
Lavery IC, 861  
Lawrence A, 1121  
Lawrence AM, 1148, 1162, 1202  
Ledgerwood AM, 836  
Lee C-S, 942  
Lee KKW, 579

Leleuc S, 207  
Lennquist S, 1141  
Lesser KL, 1127  
Levenson SM, 931  
Levine BA, 831  
Levy M, 689  
Lewin M, 452  
Lilly JR, 970  
Lin T-Y, 942  
Lindenauer SM, 472  
Linehan JH, 35  
Lipsig LJ, 616  
Little JM, 879  
Logas WG, 718  
Lo Gerfo P, 1008, 1197  
Lorentzen JE, 81  
Lucas CE, 836  
Luck SR, 677  
Luk S, 777  
Lunderquist A, 370  
Lusby RJ, 484  
Lysne J, 632

## M

Maddox AM, 54  
Maeda M, 324  
Mahoney ME, 324  
Mainwaring RD, 540  
Makarewicz PA, 700  
Malangoni MA, 648  
Malt RA, 935  
Mammen EF, 836  
Mansour EG, 708  
Marangos PJ, 1008  
Marcella KL, 72  
Mark R, 532  
Markmann DP, 251  
Marks WH, 598  
Marshall JB, 708  
Martin JK Jr, 1054  
Marx SJ, 1077  
Matas AJ, 922  
Matsuda T, 640  
Matsumoto S, 313  
Matthews DE, 405  
Mauer SM, 729  
Mavroudis C, 516  
Max MH, 845  
McBride CM, 792  
McCall A, 1202  
McCarthy HB, 68  
McCoy K, 612  
McCullough AJ, 708  
McDonald JWD, 350  
McDougal WS, 561  
McFadden D, 230  
McGhee A, 405  
McGrath PC, 135  
McNamara MF, 87  
McSweeney EM, 1045  
Meakins JL, 769  
Melliere D, 605  
Mentzer RM Jr, 540  
Merrell RC, 324  
Meyers CE, 414  
Meyers WC, 459, 907  
Michelson EL, 532  
Millikan WJ Jr, 405  
Milsom J, 121  
Minchin RF, 35  
Mitamura H, 532  
Modlin IM, 1038  
Mondschein MS, 1166  
Moon TD, 143  
Moore D, 1197  
Moore EE, 851  
Moss AA, 1083

Mozes MF, 1072  
Mule JJ, 437  
Mulvihill SJ, 500  
Murphy J, 20  
Murphy TF, 472  
Musclow CE, 1024  
Myking A, 63

## N

Nagasue N, 870  
Najarian JS, 729  
Naji A, 251  
Nashold JRB, 907  
Nathan IM, 547  
Neifeld JP, 93, 135  
Netscher DT, 158  
Newton TH, 1083  
Niederle B, 1154  
Nielsen OM, 81  
Nieuwenhuis P, 955  
Nikore V, 1135  
Nohr CW, 769  
Nolan B, 598  
Norman D, 1083  
Norton J, 1013  
Norton JA, 1077  
Nyhus LM, 619

## O

O'Dorisio T, 236  
Ogawa Y, 870  
Okerlund MD, 1083  
Opocher E, 319  
Orszulak TA, 525  
Oshima A, 213  
Oslapas R, 1031  
Ott RA, 1202

## P

Pallan TM, 976  
Palme D, 143  
Paloyan E, 1121, 1148, 1162, 1202  
Panton ONM, 20  
Pappas TN, 183  
Patel S, 927  
Patti MG, 452  
Pearce FJ, 625  
Pearce WH, 816  
Peitzman AB, 739  
Pellegrini CA, 452  
Perloff LJ, 243  
Pessa ME, 126  
Petry N, 98  
Pezzouli G, 319  
Pickleman J, 506  
Pierce GE, 824  
Pilcher WH, 344  
Pittiruti M, 378  
Placko R, 378  
Pluth JR, 525  
Pogany A, 359  
Polk HC Jr, 158  
Pollak R, 1072  
Potthoff WP, 612  
Price DC, 484  
Price JL, 12  
Price MF, 25  
Prinz RA, 1031  
Pruett TL, 371, 987 (letter)  
Pruitt BA Jr, 191

## Q

Qualy J, 760  
Quinn T, 922

Quint LE, 472  
Quarfordt PG, 484

## R

Raffensperger JG, 677  
Ramalanjaona GR, 816  
Ramphal R, 126  
Raper SE, 1000  
Rasbach DA, 1166  
Reed J, 983  
Reemtsma K, 1008  
Reilly JJ, 739  
Reilly LM, 484  
Rescorla FJ, 668  
Rettura G, 931  
Reuvers CB, 914  
Reynolds JC, 1077  
Richardson JD, 158  
Richardson S, 1008  
Rickaby DA, 35  
Rieder CF, 824  
Roberts R, 1038  
Røder OC, 81  
Rodgers BM, 121  
Rogers JF, 7  
Rohde TD, 656  
Roka R, 1154  
Rosen IB, 777, 1024, 1135, 1179  
Rosenberg SA, 437  
Rosenman JE, 816  
Rosman PM, 1127  
Rosoff L Sr, 989  
Ross JS, 935  
Rossi R, 1171  
Rotolo FS, 459  
Rotstein OD, 371, 987 (letter)  
Rubin JR, 810  
Rubio R, 540  
Rucker RD Jr, 68  
Rupp WM, 68, 656  
Rutlege R, 1107  
Ryan T, 452

## S

Sablly L, 922  
Saito S, 45  
Sakamoto T, 224  
Salam AA, 105  
Salzberg AM, 93  
Sandberg L, 1031  
Santora A, 1013  
Sauermelech C, 532  
Saunders NR, 350  
Savrin RA, 866  
Saxe A, 1031  
Saxe AW, 995, 1013  
Schafer DE, 1038  
Schaff HV, 525  
Schalm SW, 914  
Scher KS, 30  
Schirmer BD, 907  
Schnell WA Jr, 525  
Schramm W, 892  
Schraut WH, 579  
Schueneman AL, 506  
Schulak JA, 330  
Schuller HM, 35  
Schutt AJ, 1054  
Schwab W, 845  
Schwartz MZ, 430  
Schwartz SI, 344  
Scott TE, 445  
Sedgewick C, 1095  
Segadal L, 63  
Seifter E, 931  
Sganga G, 378

- Shafer RK, 143  
Shah KH, 1202  
Shah R, 927  
Shannon FL, 851  
Shapiro B, 115  
Sharp JB Jr, 158  
Shayne JP, 845  
Sheps SG, 363  
Sherlock D, 230  
Sherman JO, 677  
Shronts EP, 632  
Siegel JH, 378  
Siegel R, 98  
Silver TM, 472  
Silverman M, 1095, 1171  
Silvers WK, 251  
Simmons RL, 371, 388, 729,  
987 (letter)  
Sirinek KR, 831  
Sisson JC, 115  
Sivak MV, 746  
Sizemore GW, 363  
Skibber JM, 1077  
Smadja C, 949  
Smeds S, 1141  
Smith JA, 20  
Snover D, 68  
So SKS, 729  
Soberman R, 922  
Solberg CO, 63  
Sommer BG, 267  
Sorenson JJ, 371  
Soybel DI, 166, 174  
Spencer FC, 547  
Speyer JC, 414  
Spiegel A, 1013  
Spiegel AM, 1077  
Spiegel CA, 648  
Spina GP, 319  
Stahl WM, 976  
Stalter KD, 112  
Stanley JC, 472  
Staren ED, 718, 927  
Stark D, 1083  
Starker P, 1197  
St. Cyr JA, 396  
Steed DL, 739
- Stein TA, 784  
Stellen GP, 970  
Sterling WA Jr, 112  
Stewart MT, 105  
Stiegel M, 1107  
Stoney RJ, 484, 492  
Storozuk RB, 430  
Strauss AK, 927  
Strawbridge HTG, 1179  
Strodel WE, 1101, 1113  
Subramanian VA, 465  
Sudo K, 602  
Sugarbaker PH, 414  
Sullivan HW, 236  
Sutherland CM, 367  
Synan IS, 98  
Synn A, 500
- T
- Tache Y, 183  
Tada Y, 602  
Talpos GB, 1189  
Tanaka M, 313  
Tashjian AH Jr, 1189  
Taylor BR, 752  
Tchervenkov JI, 769  
Teasley K, 632  
Tedesco VE III, 367  
Teicher I, 784  
Tellis VA, 922  
ten Kate FWJ, 914  
Terblanche J, 1  
Terpstra OT, 914  
Theodorakis SP, 367  
Thoele S, 632  
Thomas CG Jr, 1107  
Thomas JH, 824  
Thomas LA, 760  
Thomas MJ, 452  
Thompson GB, 1054  
Thompson JC, 224, 423  
Thompson NW, 115, 1101, 1113  
Thompson RG, 810  
Tilden AB, 151  
Tilney NL, 259  
Tilson MD, 888
- Tinker MA, 784  
Tishkoff M, 1038  
Tobimatsu M, 45  
Tobin GR, 158  
Todani T, 964  
Toki A, 964  
Tomida RM, 900  
Towne JB, 799  
Townsend CM Jr, 224, 423  
Towpik E, 259  
Tribble CG, 338  
Triche T, 1013  
Tsumagari T, 45
- U
- Uemura S, 964  
Unger S, 121  
Uzzan C, 605
- V
- Valeri CR, 207  
Van Buren CT, 54  
Van Buren D, 54  
van der Lei B, 955  
Vane DW, 662  
van Heerden JA, 363, 1054  
Vargish T, 684  
Veith FJ, 922  
Verani R, 54  
Vessella RL, 143  
Viniak AI, 1101, 1113
- W
- Wait RB, 230  
Walfish PG, 1135, 1179  
Walker JP, 224, 423  
Walz DA, 836  
Wang C, 1166  
Wapnir IL, 976  
Ward CG (letter), 987  
Warren RS, 275  
Warren WD, 405  
Watanabe Y, 964  
Watne A, 684  
Watters JM, 298  
Way LW, 452
- Weakley FL, 861  
Webb LX, 12  
Webb RL, 492  
Weber C, 1197  
Weber CJ, 1008  
Webster MW, 739  
Wedel S, 378  
Weksler BB, 465  
Wells CL, 371  
Wesley JR, 598  
Wesley R, 414  
Wesley RA, 1077  
West MA, 388  
Wetstein L, 532  
Whitehouse WM Jr, 472  
Wiener I, 423  
Wild RE, 1107  
Wildevuur CRH, 955  
Wiles CE III, 378  
Williams LF Jr, 445  
Williams RA, 158  
Williams S, 879  
Wilmore DW, 291, 298  
Wise L, 784  
Wolff SM, 298  
Wool M, 1171  
Woolfitt RA, 845
- X
- Xuan GR, 313
- Y
- Yan Z-Y, 174  
Yao JST, 810  
Yoshimoto H, 313  
Younes RN, 900  
Yukaya H, 870  
Yurt RW, 191
- Z
- Zamora JL, 25  
Zannini P, 319  
Zdon MJ, 1038  
Zeldin R, 752  
Zelenock GB, 472  
Zinner MJ, 230

## SUBJECT INDEX\*

## A

**Abdomen**

- Continuous epidural infusion for analgesia after major abdominal operations: Randomized, prospective, double-blind study (Cullen et al), 718
- Chylous ascites after abdominal aortic surgery (Savrin and High), 866
- Further studies of putative cross-linking amino acid (3-deoxypyridinolone) in skin from patients with abdominal aortic aneurysms (Tilson), 888
- Microporous, compliant, biodegradable vascular grafts for regeneration of arterial wall in rat abdominal aorta (van der Lei et al), 955
- Released granulocytic elastase: Indicator of pathobiochemical alterations in septicemia after abdominal surgery (Duswald et al), 892
- Study of techniques of cardiac massage with abdomen open (Rieder et al), 824
- Treatment of intra-abdominal infections is appropriate with single-agent or combination antibiotic therapy (Malangoni, Condon, Spiegel), 648
- Use of computerized axial tomography versus peritoneal lavage in evaluation of blunt abdominal trauma: Prospective study (Davis et al), 845

**Absorption**

- Can gastrointestinal hormones enhance intestinal absorption? (Schwartz and Storozuk), 430

**Acid**

- Vitamin A and retinoic acid: Induced fibroblast differentiation in vitro (Demetriou et al), 931

**Acidosis**

- Pathophysiology of hyperchloremic metabolic acidosis after urinary diversion through intestinal segments (Koch and McDougal), 561

**Acyclovir**

- Herpes simplex burn wound infections: Epidemiology of case cluster and responses to acyclovir therapy (Brandt et al), 338

**Adenosine**

- Role of adenosine in regulation of coronary blood flow in newborn lambs (Mainwaring et al), 540

**Adrenal**

- Autotransplantation of adrenal cortical tissue: Rodent model (Saxe and Connors), 995
- Bilateral subtotal adrenal resection for bilateral pheochromocytomas in multiple endocrine neoplasia, type IIa: Case report (van Heerden et al), 363

**Adrenalectomy**

- Current status of adrenalectomy for Cushing's disease (Brunicaudi et al), 1127

**Allograft**

- Experimental pancreas allograft rejection: Correlation between histologic and functional rejection and efficacy of antirejection therapy (Schulak and Dreyvanko), 330
- Failure of canine islet allografts and autografts with cyclosporine (Merrell et al), 324
- Host-graft relationship: Systemic nature of allograft rejection (Kupiec-Weglinski et al), 259
- Portal versus systemic venous drainage for small-bowel allografts (Schraut, Abraham, Lee), 579
- Value of needle renal allograft biopsy. III. Prospective study (Matas et al), 922

**Allotransplantation**

- Parathyroid allotransplantation in treatment of complicated idiopathic primary hyperparathyroidism (Duarte et al), 1072

**Amino acid**

- Alterations in amino acid clearance during ischemia predict hepatocellular ATP changes (Fath et al), 396

July, pp. 1-130; August, pp. 131-370; September, pp. 371-618; October, pp. 619-860; November, pp. 861-988; December, pp. 989-1222.

**Amino acid, contd.**

- Further studies of putative cross-linking amino acid (3-deoxypyridinolone) in skin from patients with abdominal aortic aneurysms (Tilson), 888
- In vivo measurements of leucine metabolism with stable isotopes in normal subjects and in those with cirrhosis fed conventional and branched-chain amino acid-enriched diets (Millikan et al), 405

**Amputation**

- Management of infection of major amputation stumps after failed femorodistal grafts (Rubin et al), 810

**Analgesia**

- Continuous epidural infusion for analgesia after major abdominal operations: Randomized prospective, double-blind study (Cullen et al), 718

**Anastomosis**

- Incidence of wound infection after stapled or sutured bowel anastomosis and stapled or sutured skin closure in humans and guinea pigs (Panton et al), 20
- New technique of loop ileostomy closure after endorectal ileoanal anastomosis (Kestenbergh and Becker), 109

**Aneurysm**

- Abdominal aortic aneurysms infected by *Escherichia coli* (McNamara, Finnegan, Bakshi), 87
- Actuarial analysis of variables associated with rupture or small abdominal aortic aneurysms (Cronenwett et al), 472
- Aneurysm of persistent sciatic artery: Report of a case treated by endovascular occlusion and femoropopliteal bypass (Bequemin et al), 605
- Further studies of putative cross-linking amino acid (3-deoxypyridinolone) in skin from patients with abdominal aortic aneurysms (Tilson), 888
- Hepatic artery aneurysm associated with mucocutaneous lymph node syndrome (Marks et al), 598
- Surgical treatment of mycotic popliteal artery aneurysm: Case report and review of literature (Bonds and Fabian), 979

**Angiography**

- Angiographic demonstration of mesenteric arterial changes in postcoarctectomy syndrome (Kawauchi et al), 602

**Angioplasty**

- Septic endarteritis after percutaneous transluminal angioplasty (Krupski, Pogany, Effeney), 359

**Antagonist**

- Inhibition of human neuroblastoma by dopamine antagonists (McGrath and Neifield), 135

**Antibiotic**

- Treatment of intra-abdominal infections is appropriate with single-agent or combination antibiotic therapy (Malangoni, Condon, Spiegel), 648

**Antibody**

- Efficacy of type-specific and cross-reactive murine monoclonal antibodies directed against endotoxin during experimental sepsis (Dunn, Bogard, Cerra), 283
- Monoclonal antibodies in human renal cell carcinoma and their use in radioimmune localization and therapy of tumor xenografts (Lange et al), 143

**Antigen**

- Defective antigen presentation to a cloned T helper cell by macrophages from burned mice can be restored with interleukin-1 (Kupper et al), 199

**Aorta**

- Chylous ascites after abdominal aortic surgery (Savrin and High), 866
- Microporous, compliant, biodegradable vascular grafts for regeneration of arterial wall in rat abdominal aorta (van der Lei et al), 955
- New approach to wounds of aortic bifurcation and inferior vena cava (Salam and Stewart), 105

**Arginine hydrochloride**

- Inhibition of bile flow by intravenous arginine hydrochloride (Rotolo and Meyers), 459

**Artery**

- Angiographic demonstration of mesenteric arterial changes in postcoarctectomy syndrome (Kawauchi et al), 602
- Carotid artery thrombosis associated with lupus anticoagulant (Baker et al), 612
- Hepatic artery aneurysm associated with mucocutaneous lymph node syndrome (Marks et al), 598
- Prostacyclin production in regions of arterial stenosis (Qvarfordt et al), 484
- Surgical treatment of mycotic popliteal artery aneurysm: Case report and review of literature (Bonds and Fabian), 979

**Ascites**

- Chylous ascites after abdominal aortic surgery (Savrin and High), 866
- Effect of prostaglandin blockers on ascites fluid in pancreatitis (Farias et al), 571

**ATP**

- Alterations in amino acid clearance during ischemia predict hepatocellular ATP changes (Fath et al), 396

**Atresia**

- Intestinal atresia and stenosis: Analysis of survival in 120 cases (Rescorla and Grosfeld), 668

**Autograft**

- Failure of canine islet allografts and autografts with cyclosporine (Merrell et al), 324

**Autotransplantation**

- Autotransplantation of adrenal cortical tissue: Rodent model (Saxe and Connors), 995

**B**

**Bacteria**

- Bacterial adherence to endothelial-seeded polytetrafluoroethylene grafts (Rosenman et al), 816
- Bacterial colonization of percutaneous sutures (Gristina et al), 12
- Inhibition of povidone-iodine's bactericidal activity by common organic substances: An experimental study (Zamora et al), 25

**Betablocker**

- Betablockers compared with antithyroid drugs as preoperative treatment in hyperthyroidism: Drug tolerance, complications, and postoperative thyroid function (Lennquist et al), 1141

**$\beta$ -Endorphin**

- Pulmonary platelet trapping induced by  $\beta$ -endorphin injection in cerebrospinal fluid in dogs (Almqvist et al), 344

**Bile**

- Effects of serotonin on canine bile formation (Kortz et al), 907
- Inhibition of bile flow by intravenous arginine hydrochloride (Rotolo and Meyers), 459

**Bile duct**

- Carcinoma of extrahepatic bile ducts: Results of an aggressive surgical approach (Langer et al), 752
- Cylindrical dilatation of choledochus: Special type of congenital bile duct dilatation (Todani et al), 964
- Management of retained and recurrent bile duct stones (Broughan, Sivak, Hermann), 746
- Two approaches for electrohydraulic lithotripsy in common bile duct (Tanaka et al), 313

**Biopsy**

- Management implications from routine needle biopsy of hyperfunctioning thyroid nodules (Walsh, Strawbridge, Rosen), 1179
- Value of needle renal allograft biopsy. III. Prospective study (Matas et al), 922

**Blood**

- Influence of ultraviolet irradiation on blood transfusion effect (Balshi, Francfort, Perloff), 243

**Blood flow**

- Comparison of gastric mucosal blood flow as measured by H<sub>2</sub> gas clearance and microspheres during secretory stimulation and inhibition (Soybel et al), 174
- Role of adenosine in regulation of coronary blood flow in newborn lambs (Mainwaring et al), 540

**Bowel**

- Efficacy of polyethylene glycol-electrolyte lavage solution versus traditional mechanical bowel preparation for elective colonic surgery: Randomized, prospective, blinded clinical trial (Fleites et al), 708

**Bowel, contd.**

- Incidence of wound infection after stapled or sutured bowel anastomosis and stapled or sutured skin closure in humans and guinea pigs (Panton et al), 20
- Portal versus systemic venous drainage for small-bowel allografts (Schraut, Abraham, Lee), 579
- Prospective, randomized, double-blind study of 10% mannitol mechanical bowel preparation combined with oral neomycin and short-term, perioperative, intravenous Flagyl as prophylaxis in elective colorectal resections (Jagelman et al), 861

**Brain**

- Opposing central and peripheral actions of brain-gut peptides: Basis for regulation of gastric function (Pappas, Taché, Debas), 183

**Breast**

- Effect of operative devascularization on estrogen and progesterone receptor levels in breast cancer specimens (Teicher et al), 784
- Primary inflammatory carcinoma of the female breast: Staging and treatment possibilities (McBride and Hortobagyi), 792

**Breast cancer**

- Breast cancer in a patient with prolactinoma (Theodorakis, Tedesco, Sutherland), 367

**Budd-Chiari syndrome**

- Budd-Chiari syndrome with obstruction of the inferior caval vein: Successful treatment by cavosplenoatrial shunt (Segadal et al), 63
- Portacaval shunt in treatment of primary Budd-Chiari syndrome (Pezzouli et al), 319

**Burn**

- Herpes simplex burn wound infections: Epidemiology of case cluster and responses to acyclovir therapy (Brandt et al), 338
- Serious wound infections in burned patients (Kagan et al), 640

**Bypass**

- Aneurysm of persistent sciatic artery: Report of a case treated by endovascular occlusion and femoropopliteal bypass (Becquemin et al), 605
- Evidence for complement activation by protamine-heparin interaction after cardiopulmonary bypass (Cavarocchi et al), 525
- Functional endothelial damage by high-potassium cardioplegic solutions to saphenous vein bypass grafts (De Caterina et al), 465
- Gastritis after gastric bypass surgery (McCarthy et al), 68
- Low flow velocity predicts failure of femoropopliteal and femorotibial bypass grafts (Bandyk, Cato, Towne), 799
- Quantification of pulsatile flow during cardiopulmonary bypass to permit direct comparison of effectiveness of various types of "pulsatile" and "nonpulsatile" flow (Grossi et al), 547

**C**

**Calcium**

- Relation of serum calcium and immunoparathormone levels to parathyroid size and weight in primary hyperparathyroidism (Rutledge et al), 1107
- Serum calcium metabolism in acute experimental pancreatitis (Izquierdo et al), 1031

**Cancer**

- Breast cancer in a patient with prolactinoma (Theodorakis, Tedesco, Sutherland), 367
- Carcinoid tumors of gastrointestinal tract: Presentation, management, and prognosis (Thompson et al), 1054
- Carcinoma of extrahepatic bile ducts: Results of aggressive surgical approach (Langer et al), 752
- Effect of operative devascularization on estrogen and progesterone receptor levels in breast cancer specimens (Teicher et al), 784
- Further evidence of validity of risk group definition in differentiated thyroid carcinoma (Cady et al), 1171
- Immunotherapy of cancer with lymphokine-activated killer cells and recombinant interleukin-2 (Rosenberg and Mulé), 437
- Incidence of cancer in surgically treated thyroid nodules based on method of selection (Lo Gerfo et al), 1197
- Incidence of thyroid carcinoma in patients with Hashimoto's thyroiditis and solitary cold nodules (Ott et al), 1202
- Localization of residual medullary thyroid cancer by thallium/technetium scintigraphy (Talpos et al), 1189
- Management of nonfamilial adenomatous polyps and colon cancers (Dowling et al), 684

**Cancer, contd.**

Monoclonal antibodies in human renal cell carcinoma and their use in radioimmune localization and therapy of tumor xenografts (Lange et al), 143

Parathyroid carcinoma: Lahey Clinic experience (Cohn et al), 1095

Primary inflammatory carcinoma of the female breast: Staging and treatment possibilities (McBride and Hortobagyi), 792

Prospective, randomized trial of intravenous versus intraperitoneal 5-fluorouracil in patients with advanced primary colon or rectal cancer (Sugarbaker et al), 414

Surgical resection of segment VIII (anterosuperior subsegment of right lobe) in patients with liver cirrhosis and hepatocellular carcinoma (Franco et al), 949

Thyroid carcinoma in Graves' disease (Farbota et al), 1148

Transsternal operations in thyroid cancer (Niederle, Roka, Fritsch), 1154

**Cardiopulmonary bypass**

Evidence for complement activation by protamine-heparin interaction after cardiopulmonary bypass (Cavarocchi et al), 525

Quantification of pulsatile flow during cardiopulmonary bypass to permit direct comparison of effectiveness of various types of "pulsatile" and "nonpulsatile" flow (Grossi et al), 547

**Carotid endarterectomy**

Carotid endarterectomy in a metropolitan community: Comparison of results from three institutions (Krupski et al), 492

**Catecholamine**

Spectrum of catecholamine-secreting tumors of organ of Zuckerkandl (Altergott et al), 1121

**Celiac axis compression syndrome**

Celiac axis compression syndrome: Factors predicting favorable outcome (Williams, Gillespie, Little), 879

**Cell**

Defective antigen presentation to a cloned T helper cell by macrophages from burned mice can be restored with interleukin-1 (Kupper et al), 199

Effect of 16,16-dimethyl prostaglandin E<sub>2</sub> on gastric epithelial cell membrane potentials and resistances (Ashley, Soybel, Cheung), 166

Effects of cholecystokinin on parietal cell secretion in isolated gastric glands (Zdon et al), 1038

Hepatocyte function in sepsis: Kupffer cells mediate a biphasic protein synthesis response in hepatocytes after exposure to endotoxin or killed *Escherichia coli* (West et al), 388

Hürthle cell tumor behavior: Dilemma and resolution (Rosen, Luk, Katz), 777

Hyperplastic G cell responsiveness in vitro (Gower, McSweeney, Fabri), 1045

Immunotherapy of cancer with lymphokine-activated killer cells and recombinant interleukin-2 (Rosenberg and Mulé), 437

Morphologic and functional studies of rat hypercalcemia-associated testicular tumor maintained in cell culture (Saxe et al), 1013

**Cephalosporin**

Which cephalosporin for wound prophylaxis? An experimental comparison of three drugs (Scher and Jones), 30

**Cerebrospinal fluid**

Pulmonary platelet trapping induced by  $\beta$ -endorphin injection in cerebrospinal fluid in dogs (Almqvist et al), 344

**Chemotherapy**

Isolated total lung perfusion as a means to deliver organ-specific chemotherapy: Long-term studies in animals (Johnston et al), 35

**Children**

Effectiveness of Nissen fundoplication in neurologically impaired children with gastroesophageal reflux (Vane et al), 662

Esophageal endosclerosis in children (Stellen and Lilly), 970

Improved results of multiple renal transplantation in children (So et al), 729

Prognostic features of pediatric soft-tissue sarcomas (Neifeld et al), 93

**Cholangiography**

Prediction of operative cholangiography in patients undergoing elective cholecystectomy with routine liver function chemistries (Del Santo et al), 7

**Cholecystectomy**

Prediction of operative cholangiography in patients undergoing elective cholecystectomy with routine liver function chemistries (Del Santo et al), 7

Subtotal cholecystectomy: For the difficult gallbladder in portal hypertension and cholecystitis (Bornman and Terblanche), 1

**Cholecystitis**

Role of prostaglandins in feline experimental cholecystitis (Kaminski et al), 760

Subtotal cholecystectomy: For the difficult gallbladder in portal hypertension and cholecystitis (Bornman and Terblanche), 1

**Cholecystokinin**

Effect of aging on gallbladder contraction and release of cholecystokinin-33 in humans (Khalil et al), 423

Effects of cholecystokinin on parietal cell secretion in isolated gastric glands (Zdon et al), 1038

Gallbladder filling and response to cholecystokinin are not affected by vagotomy (Pellegrini et al), 452

**Choledochus**

Cylindrical dilatation of choledochus: Special type of congenital bile duct dilatation (Todani et al), 964

**Cholesterol**

Increases in gallbladder prostaglandin synthesis before the formation of cholesterol gallstones (LaMorte et al), 445

Reduction of plasma cholesterol and LDL-cholesterol by continuous intravenous insulin infusion (Chute et al), 656

**Cirrhosis**

In vivo measurements of leucine metabolism with stable isotopes in normal subjects and in those with cirrhosis fed conventional and branched-chain amino acid-enriched diets (Millikan et al), 405

Partial hepatectomy on cirrhotic liver with right lateral tumor (Lee, Chao, Lin), 942

Surgical resection of segment VIII (anterosuperior subsegment of right lobe) in patients with liver cirrhosis and hepatocellular carcinoma (Franco et al), 949

**Citizenship**

Presidential address: Cost of academic citizenship (SUS) (Flint), 131

**Colitis**

Experience with endorectal pull-through and S pouch for ulcerative colitis and familial polyposis in adults (Bubrick, Jacobs, Levy), 689

**Colon**

Efficacy of polyethylene glycol-electrolyte lavage solution versus traditional mechanical bowel preparation for elective colonic surgery: Randomized, prospective, blinded clinical trial (Fleites et al), 708

Management of nonfamilial adenomatous polyps and colon cancers (Dowling et al), 684

Primary repair of colon: When is it safe alternative? (Shannon and Moore), 851

Prospective, randomized, double-blind study combined with oral neomycin and short-term, perioperative, intravenous Flagyl as prophylaxis in elective colorectal resections (Jagelman et al), 861

Prospective, randomized trial of intravenous versus intraperitoneal 5-fluorouracil in patients with advanced primary colon or rectal cancer (Sugarbaker et al), 414

**Cortex**

Autotransplantation of adrenal cortical tissue: Rodent model (Saxe and Connors), 995

**Cost**

Presidential address: Cost of academic citizenship (SUS) (Flint), 131

**Culture**

Morphologic and functional studies of rat hypercalcemia-associated testicular tumor maintained in cell culture (Saxe et al), 1013

**Cushing's disease**

Current status of adrenalectomy for Cushing's disease (Brunnicardi et al), 1127

**Cyclosporine**

De novo hemolytic uremic syndrome in renal transplant recipients immunosuppressed with cyclosporine (Van Buren et al), 54

**Cyclosporine, contd.**

Failure of canine islet allografts and autografts with cyclosporine (Merrell et al), 324

**Cytotoxicity**

Cellular immune defects in patients with melanoma involving interleukin-2-activated lymphocyte cytotoxicity and serum suppressor factor (Balch, Itoh, Tilden), 151

**D**

**3-Deoxyypyridinoline**

Further studies of putative cross-linking amino acid (3-deoxyypyridinoline) in skin from patients with abdominal aortic aneurysms (Tilson), 888

**Devascularization**

Effect of operative devascularization on estrogen and progesterone receptor levels in breast cancer specimens (Teicher et al), 784

**Diabetes**

"Activated" T-lymphocyte levels in spontaneously diabetic BB rat syndrome (Francfort et al), 251

**Dilatation**

Cylindrical dilatation of choledochus: Special type of congenital bile duct dilatation (Todani et al), 963

**16,16-Dimethyl prostaglandin E<sub>2</sub>**

Effect of 16,16-dimethyl prostaglandin E<sub>2</sub> on gastric epithelial cell membrane potentials and resistances (Ashley, Soybel, Cheung), 166

**DNA**

DNA histogram of parathyroid tissue in determining extent of parathyroidectomy (Rosen and Musclove), 1024

**Donor**

Mismatched living, related donor renal transplantation: Prospective, randomized study (Sommer and Ferguson), 267

**Dopamine**

Inhibition of human neuroblastoma by dopamine antagonists (McGrath and Neifield), 135

**Drainage**

Portal versus systemic venous drainage for small-bowel allografts (Schraut, Abraham, Lee), 579

**Drug**

Betablockers compared with antithyroid drugs as preoperative treatment in hyperthyroidism: Drug tolerance, complications, and postoperative thyroid function (Lennquist et al), 1141

**E**

**Elastase**

Released granulocytic elastase: Indicator of pathobiochemical alterations in septicemia after abdominal surgery (Duswald et al), 892

**Embolism**

Indium 111 platelet imaging for detection of deep venous thrombosis and pulmonary embolism in patients without symptoms after surgery (Clarke-Pearson et al), 98

**Enderterectomy**

Carotid endarterectomy in a metropolitan community: Comparison of results from three institutions (Krupski et al), 492

**Enderteritis**

Septic endarteritis after percutaneous transluminal angioplasty (Krupski, Pogany, Effeney), 359

**Endosclerosis**

Esophageal endosclerosis in children (Stellen and Lilly), 970

**Endothelium**

Bacterial adherence to endothelial-seeded polytetrafluoroethylene grafts (Rosenman et al), 816

Functional endothelial damage by high-potassium cardioplegic solutions to saphenous vein bypass grafts (De Caterina et al), 465

**Endotoxin**

Efficacy of type-specific and cross-reactive murine monoclonal antibodies directed against endotoxin during experimental sepsis (Dunn, Bogard, Cerra), 283

Hepatocyte function in sepsis: Kupffer cells mediate a biphasic protein synthesis response in hepatocytes after exposure to endotoxin or killed *Escherichia coli* (West et al), 388

**Enolase**

Presence of neuron-specific enolase and somatostatin in human parathyroid tissues (Weber et al), 1008

**Epidermis**

Epidermal growth factor receptors in normal and neoplastic thyroid tissue (Duh et al), 1000

**Epithelium**

Epithelial lining methods in esophageal repair: A comparative study using pedicle flaps in cats (Tobin et al), 158

**Escherichia coli**

Abdominal aortic aneurysms infected by *Escherichia coli* (McNamara, Finnegan, Bakshi), 87

Hepatocyte function in sepsis: Kupffer cells mediate a biphasic protein synthesis response in hepatocytes after exposure to endotoxin or killed *Escherichia coli* (West et al), 388

In vivo growth of *Escherichia coli* (letter) (Ward), 987; Reply (Pruett, Rotstein, Simmons), 987

Limited effects of prostaglandin inhibitors in *Escherichia coli* sepsis (Hulton, Johnson, Wilmore), 291

Mechanism of adjuvant effect of hemoglobin in experimental peritonitis IX: Infection-potentiating effect of hemoglobin in *Escherichia coli* peritonitis is strain specific (Pruett et al), 371

**Esophageal atresia**

Temporary banding of gastroesophageal junction in critically ill neonate with esophageal atresia and tracheoesophageal fistula (Fagelman and Boyarsky), 594

**Esophagus**

Epithelial lining methods in esophageal repair: Comparative study using pedicle flaps in cats (Tobin et al), 158

TriPLICATION of esophagus with gastric duplication (Milsom et al), 121

**Estrogen**

Effect of operative devascularization on estrogen and progesterone receptor levels in breast cancer specimens (Teicher et al), 784

**F**

**Fat**

Importance of ileum in neurotensin released by fat (Walker et al), 224

**Feeding**

Enteral feeding in sepsis: Prospective, randomized, double-blind trial (Cerra et al), 632

In utero supplemental fetal feeding in an animal model: Effects of fetal growth and development (Mulvihill et al), 500

**Fetus**

In utero supplemental fetal feeding in an animal model: Effects of fetal growth and development (Mulvihill et al), 500

**Fibroblast**

Vitamin A and retinoic acid: Induced fibroblast differentiation in vitro (Demetriou et al), 931

**Fistula**

Temporary banding of gastroesophageal junction in critically ill neonate with esophageal atresia and tracheoesophageal fistula (Fagelman and Boyarsky), 594

**Flagyl**

Prospective, randomized, double-blind study combined with oral neomycin and short-term, perioperative, intravenous Flagyl as prophylaxis in elective colorectal resections (Jagelman et al), 861

**5-Flourouracil**

Prospective, randomized trial of intravenous versus intraperitoneal 5-flourouracil in patients with advanced primary colon or rectal cancer (Sugarbaker et al), 414

**Flow**

Quantification of pulsatile flow during cardiopulmonary bypass to permit direct comparison of effectiveness of various types of "pulsatile" and "nonpulsatile" flow (Grossi et al), 547

**Food**

Surgical sympathectomy increases pancreatic polypeptide response to food (Larson, Sullivan, O'Dorisio), 236

**Frozen section**

Declining role of frozen section in surgical planning for thyroid nodules (Hamburger and Hamburger), 307

**G**

**Gallbladder**

Effect of aging on gallbladder contraction and release of cholecystokinin-33 in humans (Khalil et al), 423

**Gallbladder, contd.**

- Gallbladder filling and response to cholecystokinin are not affected by vagotomy (Pellegrini et al), 452
- Increases in gallbladder prostaglandin synthesis before formation of cholesterol gallstones (LaMorte et al), 445
- Subtotal cholecystectomy: For difficult gallbladder in portal hypertension and cholecystitis (Bornman and Terblanche), 1

**Gastric duplication**

- Triplication of esophagus with gastric duplication (Milsom et al), 121

**Gastrinoma**

- Extrapaneatic gastrinomas (Thompson et al), 1113

**Gastritis**

- Gastritis after gastric bypass surgery (McCarthy et al), 68

**Gastroesophageal reflux**

- Effectiveness of Nissen fundoplication in neurologically impaired children with gastroesophageal reflux (Vane et al), 662

**Gastrointestinal tract**

- Carcinoid tumors of gastrointestinal tract: Presentation, management, and prognosis (Thompson et al), 1054

**Gastroplasty**

- Vertical banded gastroplasty: Assessment of efficacy (Makarewicz et al), 700

**Gastroschisis**

- Gastroschisis in 106 consecutive newborn infants (Luck et al), 677

**G cell**

- Hyperplastic G cell responsiveness in vitro (Gower, McSweeney, Fabri), 1045

**Gland**

- Effects of cholecystokinin on parietal cell secretion in isolated gastric glands (Zdon et al), 1038

**Graft**

- Bacterial adherence to endothelial-seeded polytetrafluoroethylene grafts (Rosenman et al), 816
- Functional endothelial damage by high-potassium cardioplegic solutions to saphenous vein bypass grafts (De Caterina et al), 465
- Host-graft relationship: Systemic nature of allograft rejection (Kupiec-Weglinski et al), 259
- Low flow velocity predicts failure of femoropopliteal and femorotibial bypass grafts (Bandyk, Cato, Towne), 799
- Management of infection of major amputation stumps after failed femorodistal grafts (Rubin et al), 810
- Microporous, compliant, biodegradable vascular grafts for regeneration of arterial wall in rat abdominal aorta (van der Lei et al), 955
- Vascular graft infection: Analysis of sixty-two graft infections in 2411 consecutively implanted synthetic vascular grafts (Lorentzen et al), 81

**Graves' disease**

- Thyroid carcinoma in Graves' disease (Farbota et al), 1148

**Growth**

- Epidermal growth factor receptors in normal and neoplastic thyroid tissue (Duh et al), 1000

**H****Hashimoto's thyroiditis**

- Incidence of thyroid carcinoma in patients with Hashimoto's thyroiditis and solitary cold nodules (Ott et al), 1202

**Heart**

- Control of gastric vascular resistance in cardiogenic shock (Bulkley et al), 213
- General surgical complications in heart and heart-lung transplantation (Steed et al), 739
- Role of adenosine in regulation of coronary blood flow in newborn lambs (Mainwaring et al), 540
- Study of techniques of cardiac massage with abdomen open (Rieder et al), 824

**Hemodynamic effect**

- Hemodynamic effects of surface cooling-induced hypothermia on immature pigs with ventricular septal defects (Ganzel et al), 516

**Hemoglobin**

- Mechanism of adjuvant effect of hemoglobin in experimental peritonitis IX: Infection-potentiating effect of hemoglobin in *Escherichia coli* peritonitis is strain specific (Pruett et al), 371

**Hemolytic uremic syndrome**

- De novo hemolytic uremic syndrome in renal transplant recipients immunosuppressed with cyclosporine (Van Buren et al), 54

**Hemorrhage**

- Hepatic subcapsular hemorrhage associated with pregnancy (Stalter and Sterling), 112

**Hemostasis**

- Serial changes in primary hemostasis after massive transfusion (Harrigan et al), 836

**Heparin**

- Evidence for complement activation by protamine-heparin interaction after cardiopulmonary bypass (Cavarocchi et al), 525

**Hepatectomy**

- Partial hepatectomy on cirrhotic liver with right lateral tumor (Lee, Chao, Lin), 942

**Hepatocyte**

- Hepatocyte function in sepsis: Kupffer cells mediate a biphasic protein synthesis response in hepatocytes after exposure to endotoxin or killed *Escherichia coli* (West et al), 388
- Protein synthesis in tumor-influenced hepatocyte (Warren, Jeevanandam, Brennan), 275

**Herpes simplex**

- Herpes simplex burn wound infections: Epidemiology of case cluster and responses to acyclovir therapy (Brandt et al), 338

**H<sub>2</sub> gas clearance**

- Comparison of gastric mucosal blood flow as measured by H<sub>2</sub> gas clearance and microspheres during secretory stimulation and inhibition (Soybel et al), 174

**Histopathology**

- Histopathologic factors conducive to experimental ventricular tachycardia (Wetstein et al), 532

**Hormone**

- Can gastrointestinal hormones enhance intestinal absorption? (Schwartz and Storozuk), 430
- Hyperparathyroidism and gastroenteropancreatic hormone levels (Strodel et al), 1101

**Host**

- Host-graft relationship: Systemic nature of allograft rejection (Kupiec-Weglinski et al), 259

**Hürthle cell**

- Hürthle cell tumor behavior: Dilemma and resolution (Rosen, Luk, Katz), 777
- Hürthle cell tumors of thyroid gland (Heppe et al), 1162

**Hypercalcemia**

- Morphologic and functional studies of rat hypercalcemia-associated testicular tumor maintained in cell culture (Saxe et al), 1013

**Hypergraphia**

- Presidential address: Hyperparathyroidism, hypergraphia, and just plain hype (AAES) (Rosoff), 989

**Hyperparathyroidism**

- Hyperparathyroidism and gastroenteropancreatic hormone levels (Strodel et al), 1101
- Localization studies in patients with persistent or recurrent hyperparathyroidism (Clark et al), 1083
- Observations on course on untreated primary hyperparathyroidism (Corlew et al), 1064
- Presidential address: Hyperparathyroidism, hypergraphia, and just plain hype (AAES) (Rosoff), 989
- Relation of serum calcium and immunoparathormone levels to parathyroid size and weight in primary hyperparathyroidism (Rutledge et al), 1107

**Hypertension**

- Subtotal cholecystectomy: For difficult gallbladder in portal hypertension and cholecystitis (Bornman and Terblanche), 1

**Hyperthyroidism**

- Betablockers compared with antithyroid drugs as preoperative treatment in hyperthyroidism: Drug tolerance, complications, and postoperative thyroid function (Lennquist et al), 1141

**Hypoparathyroidism**

- Parathyroid allotransplantation in treatment of complicated idiopathic primary hyperparathyroidism (Duarte et al), 1072

**Hypothermia**

- Hemodynamic effects of surface cooling-induced hypothermia on immature pigs with ventricular septal defects (Ganzel et al), 516

I

- Ileostomy**  
Assessment of an implantable ileostomy sphincter (Chandler et al), 72  
New technique of loop ileostomy closure after endorectal ileoanal anastomosis (Kestenberg and Becker), 109
- Ileum**  
Effect of luminal contents on postresectional longitudinal and mucosal growth in ileum of suckling rats (Ford et al), 935  
Importance of ileum in neurotensin released by fat (Walker et al), 224
- Imaging**  
Indium 111 platelet imaging for detection of deep venous thrombosis and pulmonary embolism in patients without symptoms after surgery (Clarke-Pearson et al), 98
- Immunity**  
Malnutrition and humoral immunity: Short-term acute nutritional deprivation (Nohr et al), 769
- Immunology**  
Spontaneous lymphocyte activity: Important but neglected component of immunologic profile of thermally injured patient (Deitch), 587
- Immunoparathormone**  
Relation of serum calcium and immunoparathormone levels to parathyroid size and weight in primary hyperparathyroidism (Rutledge et al), 1107
- Immunotherapy**  
Immunotherapy of cancer with lymphokine-activated killer cells and recombinant interleukin-2 (Rosenberg and Mulé), 437
- Indium 111**  
Indium 111 platelet imaging for detection of deep venous thrombosis and pulmonary embolism in patients without symptoms after surgery (Clarke-Pearson et al), 98
- Infant**  
Gastroschisis in 106 consecutive newborn infants (Luck et al), 677
- Infection**  
Decreased wound neutrophils and indiscrete margination in pathogenesis of wound infection (Yurt and Pruitt), 191  
Herpes simplex burn wound infections: Epidemiology of case cluster and responses to acyclovir therapy (Brandt et al), 338  
Incidence of wound infection after stapled or sutured bowel anastomosis and stapled or sutured skin closure in humans and guinea pigs (Panton et al), 20  
Management of infection of major amputation stumps after failed femorodistal grafts (Rubin et al), 810  
Mechanism of adjuvant effect of hemoglobin in experimental peritonitis IX: Infection-potentiating effect of hemoglobin in *Escherichia coli* peritonitis is strain specific (Pruett et al), 371  
Necrotizing soft-tissue infections caused by marine vibrios (Howard et al), 126  
Serious wound infections in burned patients (Kagan et al), 640  
Treatment of intra-abdominal infections is appropriate with single-agent or combination antibiotic therapy (Malangoni, Condon, Spiegel), 648  
Vascular graft infection: An analysis of sixty-two graft infections in 2411 consecutively implanted synthetic vascular grafts (Lorentzen et al), 81
- Infusion**  
Continuous epidural infusion for analgesia after major abdominal operations: Randomized, prospective, double-blind study (Cullen et al), 718  
Reduction of plasma cholesterol and LDL-cholesterol by continuous intravenous insulin infusion (Chute et al), 656
- Injection corrosion**  
Injection corrosion technique (letter) (Gans), 370; Reply (Lunderquist), 370
- Injury**  
Contribution of circulating formed elements to prostanoid production in complement-mediated lung injury in sheep (Egan et al), 350  
Spontaneous lymphocyte activity: Important but neglected component of immunologic profile of thermally injured patient (Deitch), 587  
Traumatic injury to proximal superior mesenteric vessels (Sirinek and Levine), 831

Insulin

- Reduction of plasma cholesterol and LDL-cholesterol by continuous intravenous insulin infusion (Chute et al), 656
- Interleukin-1**  
Defective antigen presentation to a cloned T helper cell by macrophages from burned mice can be restored with interleukin-1 (Kupper et al), 199  
Induction of interleukin-1 in humans and its metabolic effects (Watters et al), 298
- Interleukin-2**  
Cellular immune defects in patients with melanoma involving interleukin-2-activated lymphocyte cytotoxicity and serum suppressor factor (Balch, Itoh, Tilden), 151  
Immunotherapy of cancer with lymphokine-activated killer cells and recombinant interleukin-2 (Rosenberg and Mulé), 437
- Intestine**  
Can gastrointestinal hormones enhance intestinal absorption? (Schwartz and Storozuk), 430  
Verapamil inhibition of intestinal effects of substance P (Zinner et al), 230
- Irradiation**  
Influence of ultraviolet irradiation on blood transfusion effect (Balshi, Francfort, Perloff), 243
- Ischemia**  
Alterations in amino acid clearance during ischemia predict hepatocellular ATP changes (Fath et al), 396  
Prostacyclin and thromboxane A<sub>2</sub> moderate posts ischemic renal failure (Lelcuk et al), 207  
Protective effect of prostaglandin E<sub>1</sub> on ischemia-induced acute renal failure in dogs (Tobimatsu et al), 45
- Isotope**  
In vivo measurements of leucine metabolism with stable isotopes in normal subjects and in those with cirrhosis fed conventional and branched-chain amino acid-enriched diets (Millikan et al), 405

K

Kidney

- De novo hemolytic uremic syndrome in renal transplant recipients immunosuppressed with cyclosporine (Van Buren et al), 54  
Improved results of multiple renal transplantation in children (So et al), 729  
Mismatched living, related donor renal transplantation: Prospective, randomized study (Sommer and Ferguson), 267  
Monoclonal antibodies in human renal cell carcinoma and their use in radioimmune localization and therapy of tumor xenografts (Lange et al), 143  
Prostacyclin and thromboxane A<sub>2</sub> moderate posts ischemic renal failure (Lelcuk et al), 207  
Protective effect of prostaglandin E<sub>1</sub> on ischemia-induced acute renal failure in dogs (Tobimatsu et al), 45  
Value of needle renal allograft biopsy. III. Prospective study (Matas et al), 922
- Kupffer cell**  
Hepatocyte function in sepsis: Kupffer cells mediate a biphasic protein synthesis response in hepatocytes after exposure to endotoxin or killed *Escherichia coli* (West et al), 388

L

Lactate

- Extracellular-intracellular lactate gradients in skeletal muscle during hemorrhagic shock in rat (Pearce, Connett, Drucker), 625
- Lahey Clinic**  
Parathyroid carcinoma: Lahey Clinic experience (Cohn et al), 1095
- Lavage**  
Efficacy of polyethylene glycol-electrolyte lavage solution versus traditional mechanical bowel preparation for elective colonic surgery: Randomized, prospective, blinded clinical trial (Fleites et al), 708  
Use of computerized axial tomography versus peritoneal lavage in evaluation of blunt abdominal trauma: Prospective study (Davis et al), 845
- Leucine**  
Increased dependence of leucine in posttraumatic sepsis: Leucine/tyrosine clearance ratio as indicator of hepatic impairment in septic multiple organ failure syndrome (Pittiruti et al), 378

**Leucine, contd.**

In vivo measurement of leucine metabolism with stable isotopes in normal subjects and in those with cirrhosis fed conventional and branched-chain amino acid-enriched diets (Millikan et al), 405

**Lithium**

Postoperative thyroid storm after lithium preparation (Reed and Bradley), 983

**Lithotripsy**

Two approaches for electrohydraulic lithotripsy in common bile duct (Tanaka et al), 313

**Liver**

Auxiliary transplantation of part of liver improves survival and provides metabolic support in pigs with acute liver failure (Reuvers et al), 914

Hepatic subcapsular hemorrhage associated with pregnancy (Stalter and Sterling), 112

Increased dependence of leucine in posttraumatic sepsis: Leucine/tyrosine clearance ratio as indicator of hepatic impairment in septic multiple organ failure syndrome (Pittiruti et al), 378

Partial hepatectomy on cirrhotic liver with right lateral tumor (Lee, Chao, Lin), 942

Prediction of operative cholangiography in patients undergoing elective cholecystectomy with routine liver function chemistries (Del Santo et al), 7

Surgical resection of segment VIII (anterosuperior subsegment of right lobe) in patients with liver cirrhosis and hepatocellular carcinoma (Franco et al), 949

**Localization**

Localization of residual medullary thyroid cancer by thallium/technetium scintigraphy (Talpos et al), 1189

Localization studies in patients with persistent or recurrent hyperparathyroidism (Clark et al), 1083

Monoclonal antibodies in human renal cell carcinoma and their use in radioimmune localization and therapy of tumor xenografts (Lange et al), 143

**Lumen**

Effect of luminal contents on postresectional longitudinal and mucosal growth in ileum of suckling rats (Ford et al), 935

**Lung**

Contribution of circulating formed elements to prostanoid production in complement-mediated lung injury in sheep (Egan et al), 350

General surgical complications in heart and heart-lung transplantation (Steed et al), 739

Isolated total lung perfusion as means to deliver organ-specific chemotherapy: Long-term studies in animals (Johnston et al), 35

Pulmonary platelet trapping induced by  $\beta$ -endorphin injection in cerebrospinal fluid in dogs (Almqvist et al), 344

Role of lung innervation in hemodynamic response to hypertonic sodium chloride solutions in hemorrhagic shock (Younes et al), 900

**Lupus anticoagulant**

Carotid artery thrombosis associated with lupus anticoagulant (Baker et al), 612

**Lymphocyte**

Cellular immune defects in patients with melanoma involving interleukin-2-activated lymphocyte cytotoxicity and serum suppressor factor (Balch, Itoh, Tilden), 151

Spontaneous lymphocyte activity: Important but neglected component of immunologic profile of thermally injured patient (Deitch), 587

**Lymphokine**

Immunotherapy of cancer with lymphokine-activated killer cells and recombinant interleukin-2 (Rosenberg and Mulé), 437

**Lymphoma**

Malignant lymphoma of thyroid gland: Clinical and pathologic study of twenty cases (Rasbach et al), 1166

**M****Macrophage**

Defective antigen presentation to cloned T helper cell by macrophages from burned mice can be restored with interleukin-1 (Kupper et al), 199

**Malnutrition**

Malnutrition and humoral immunity: Short-term acute nutritional deprivation (Nohr et al), 769

**Mannitol**

Prospective, randomized, double-blind study of 10% mannitol mechanical bowel preparation combined with oral neomycin and short-term, perioperative, intravenous Flagyl as prophylaxis in elective colorectal resections (Jagelman et al), 861

**Margination**

Decreased wound neutrophils and indiscrete margination in pathogenesis of wound infection (Yurt and Pruitt), 191

**Massage**

Study of techniques of cardiac massage with abdomen open (Rieder et al), 824

**Melanoma**

Cellular immune defects in patients with melanoma involving interleukin-2-activated lymphocyte cytotoxicity and serum suppressor factor (Balch, Itoh, Tilden), 151

**Metabolism**

Auxiliary transplantation of part of liver improves survival and provides metabolic support in pigs with acute liver failure (Reuvers et al), 914

Induction of interleukin-1 in humans and its effects (Watters et al), 298

In vivo measurements of leucine metabolism with stable isotopes in normal subjects and in those with cirrhosis fed conventional and branched-chain amino acid-enriched diets (Millikan et al), 405

Serum calcium metabolism in acute experimental pancreatitis (Izquierdo et al), 1031

**Microsphere**

Comparison of gastric mucosal blood flow as measured by H<sub>2</sub> gas clearance and microspheres during secretory stimulation and inhibition (Soybel et al), 174

**Mucocutaneous lymph node syndrome**

Hepatic artery aneurysms associated with mucocutaneous lymph node syndrome (Marks et al), 598

**Multiple endocrine neoplasia**

Bilateral subtotal adrenal resection for bilateral pheochromocytomas in multiple endocrine neoplasia, type IIa: Case report (van Heerden et al), 363

**Multiple organ failure syndrome**

Increased dependence of leucine in posttraumatic sepsis: Leucine/tyrosine clearance ratio as an indicator of hepatic impairment in septic multiple organ failure syndrome (Pittiruti et al), 378

**Muscle**

Extracellular-intracellular lactate gradients in skeletal muscle during hemorrhage shock in the rat (Pearce, Connert, Drucker), 625

**N****Neomycin**

Prospective, randomized, double-blind study of 10% mannitol mechanical bowel preparation combined with oral neomycin and short-term, perioperative, intravenous Flagyl as prophylaxis in elective colorectal resections (Jagelman et al), 861

**Neuroblastoma**

Inhibition of human neuroblastoma by dopamine antagonists (McGrath and Neifield), 135

**Neurotensin**

Importance of ileum in neurotensin released by fat (Walker et al), 224

**Neutrophil**

Decreased wound neutrophils and indiscrete margination in pathogenesis of wound infection (Yurt and Pruitt), 191

**Nissen fundoplication**

Effectiveness of Nissen fundoplication in neurologically impaired children with gastroesophageal reflux (Vane et al), 662

**Nodule**

Incidence of cancer in surgically treated thyroid nodules based on method of selection (Lo Gerfo et al), 1197

Incidence of thyroid carcinoma in patients with Hashimoto's thyroiditis and solitary cold nodules (Ott et al), 1202

Management implications from routine needle biopsy of hyperfunctioning thyroid nodules (Walfish, Strawbridge, Rosen), 1179

**Nutrition**

Malnutrition and humoral immunity: Short-term acute nutritional deprivation (Nohr et al), 769

**O**

**Obstruction**

Budd-Chiari syndrome with obstruction of the inferior caval vein: Successful treatment by cavosplenoatrial shunt (Segadal et al), 63

**Occlusion**

Aneurysm of persistent sciatic artery: Report of a case treated by endovascular occlusion and femoropopliteal bypass (Becquemin et al), 605

**Operative skill**

Age, gender, lateral dominance, and prediction of operative skill among general surgery residents (Schueneman, Pickleman, Freeark), 506

**P**

**Pancreas**

Experimental pancreas allograft rejection: Correlation between histologic and functional rejection and efficacy of antirejection therapy (Schulak and Drevyanko), 330

Extrapancratic gastrinomas (Thompson et al), 1113

Surgical sympathectomy increases pancreatic polypeptide response to food (Larson, Sullivan, O'Dorisio), 236

**Pancreatitis**

Effect of prostaglandin blockers on ascites fluid in pancreatitis (Farias et al), 571

Serum calcium metabolism in acute experimental pancreatitis (Izquierdo et al), 1031

**Parathyroid**

Computerized technetium/thallium scans and parathyroid reoperation (Skibber et al), 1077

DNA histogram of parathyroid tissue in determining extent of parathyroidectomy (Rosen and Musclow), 1024

Parathyroid allotransplantation in treatment of complicated idiopathic primary hyperparathyroidism (Duarte et al), 1072

Parathyroid carcinoma: Lahey Clinic experience (Cohn et al), 1095

Presence of neuron-specific enolase and somatostatin in human parathyroid tissues (Weber et al), 1008

Relation of serum calcium and immunoparathormone levels to parathyroid size and weight in primary hyperparathyroidism (Rutledge et al), 1107

**Pedicle flap**

Epithelial lining methods in esophageal repair: Comparative study using pedicle flaps in cats (Tobin et al), 158

**Peptide**

Opposing central and peripheral actions of brain-gut peptides: Basis for regulation of gastric function (Pappas, Taché, Debas), 183

**Perfusion**

Isolated total lung perfusion as a means to deliver organ-specific chemotherapy: Long-term studies in animals (Johnston et al), 35

**Peritonitis**

Mechanism of adjuvant effect of hemoglobin in experimental peritonitis IX: Infection-potentiating effect of hemoglobin in *Escherichia coli* peritonitis is strain specific (Pruett et al), 371

**Pheochromocytoma**

Bilateral subtotal adrenal resection for bilateral pheochromocytomas in multiple endocrine neoplasia, type IIa: Case report (van Heerden et al), 363

Limitations of <sup>131</sup>I-MIBG scintigraphy in locating pheochromocytomas (Gough et al), 115

Pheochromocytoma: Operative strategy (Cullen et al), 927

**Platelet**

Pulmonary platelet trapping induced by  $\beta$ -endorphin injection in cerebrospinal fluid in dogs (Almqvist et al), 344

**Polyp**

Management of nonfamilial adenomatous polyps and colon cancers (Dowling et al), 684

**Polypeptide**

Surgical sympathectomy increases pancreatic polypeptide response to food (Larson, Sullivan, O'Dorisio), 236

**Polyposis**

Experience with endorectal pull-through and S pouch for ulcerative colitis and familial polyposis in adults (Bubrick, Jacobs, Levy), 689

**Polytetrafluoroethylene**

Bacterial adherence to endothelial-seeded polytetrafluoroethylene grafts (Rosenman et al), 816

Modified distal splenorenal shunt with expanded polytetrafluoroethylene interposition (Nagasue et al), 870

**Postcoarctectomy syndrome**

Angiographic demonstration of mesenteric arterial changes in postcoarctectomy syndrome (Kawauchi et al), 602

**Potassium**

Functional endothelial damage by high-potassium cardioplegic solutions to saphenous vein bypass grafts (De Caterina et al), 465

**Pouch**

Experience with endorectal pull-through and S pouch for ulcerative colitis and familial polyposis in adults (Bubrick, Jacobs, Levy), 689

**Povidone-iodine**

Inhibition of povidone-iodine's bactericidal activity by common organic substances: An experimental study (Zamora et al), 25

**Pregnancy**

Hepatic subcapsular hemorrhage associated with pregnancy (Stalter and Sterling), 112

Pregnancy and surgical thyroid disease (Rosen, Walfish, Nikore), 1135

**Presidential address**

Presidential address: Academic surgery—Points of View (CSA) (Nyhus), 619

Presidential address: Cost of academic citizenship (SUS) (Flint), 131

Presidential address: Hyperparathyroidism, hypergraphia, and just plain hype (AAES) (Rosoff), 989

**Progesterone**

Effect of operative devascularization on estrogen and progesterone receptor levels in breast cancer specimens (Teicher et al), 784

**Prolactinoma**

Breast cancer in a patient with prolactinoma (Theodorakis, Tedesco, Sutherland), 367

**Prophylaxis**

Which cephalosporin for wound prophylaxis? An experimental comparison of three drugs (Scher and Jones), 30

**Prostacyclin**

Prostacyclin production in regions of arterial stenosis (Qvarfordt et al), 484

Prostacyclin and thromboxane A<sub>2</sub> moderate postischemic renal failure (Lelcuk et al), 207

**Prostaglandin**

Effect of 16,16-dimethyl prostaglandin E<sub>2</sub> on gastric epithelial cell membrane potentials and resistances (Ashley, Soybel, Cheung), 166

Effect of prostaglandin blockers on ascites fluid in pancreatitis (Farias et al), 571

Increases in gallbladder prostaglandin synthesis before the formation of cholesterol gallstones (LaMorte et al), 445

Limited effects of prostaglandin inhibitors in *Escherichia coli* sepsis (Hulton, Johnson, Wilmore), 291

Protective effect of prostaglandin E<sub>1</sub> on ischemia-induced acute renal failure in dogs (Tobimatsu et al), 45

Role of prostaglandins in feline experimental cholecystitis (Kaminski et al), 760

**Prostanoid**

Contribution of circulating formed elements to prostanoid production in complement-mediated lung injury in sheep (Egan et al), 350

**Protamine**

Evidence for complement activation by protamine-heparin interaction after cardiopulmonary bypass (Cavarocchi et al), 525

**Protein**

Hepatocyte function in sepsis: Kupffer cells mediate a biphasic protein synthesis response in hepatocytes after exposure to endotoxin or killed *Escherichia coli* (West et al), 388

Protein synthesis in tumor-influenced hepatocyte (Warren, Jeevanandam, Brennan), 275

**Pulsatile flow**

Quantification of pulsatile flow during cardiopulmonary bypass to permit direct comparison of effectiveness of various types of "pulsatile" and "nonpulsatile" flow (Grossi et al), 547

**R****Rectum**

Prospective, randomized trial of intravenous versus intraperitoneal 5-fluorouracil in patients with advanced primary colon or rectal cancer (Sugarbaker et al), 414

**Regeneration**

Microporous, compliant, biodegradable vascular grafts for regeneration of arterial wall in rat abdominal aorta (van der Lei et al), 955

**Relaparotomy**

Urgent relaparotomy: High-risk, no-choice operation (Bunt), 555

**Resident**

Age, gender, lateral dominance, and prediction of operative skill among general surgery residents (Schueneman, Pickleman, Freeark), 506

**Risk group**

Further evidence of validity of risk group definition in differentiated thyroid carcinoma (Cady et al), 1171

**Rupture**

Actuarial analysis of variables associated with rupture of small abdominal aortic aneurysms (Cronenwett et al), 472

**S****Sarcoma**

Prognostic features of pediatric soft-tissue sarcomas (Neifeld et al), 93

**Scan**

Computerized technetium/thallium scans and parathyroid reoperation (Skibber et al), 1077

**Sciatic artery**

Aneurysm of persistent sciatic artery: Report of a case treated by endovascular occlusion and femoropopliteal bypass (Becquemini et al), 605

**Scintigraphy**

Limitations of <sup>131</sup>I-MIBG scintigraphy in locating pheochromocytomas (Gough et al), 115

Localization of residual medullary thyroid cancer by thallium/technetium scintigraphy (Talops et al), 1189

**Secretion**

Effects of cholecystokinin on parietal cell secretion in isolated gastric glands (Zdon et al), 1038

**Sepsis**

Efficacy of type-specific and cross-reactive murine monoclonal antibodies directed against endotoxin during experimental sepsis (Dunn, Bogard, Cerra), 283

Enteral feeding in sepsis: Prospective, randomized, double-blind trial (Cerra et al), 632

Hepatocyte function in sepsis: Kupffer cells mediate a biphasic protein synthesis response in hepatocytes after exposure to endotoxin or killed *Escherichia coli* (West et al), 388

Increased dependence of leucine in posttraumatic sepsis: Leucine/tyrosine clearance ratio as an indicator of hepatic impairment in septic multiple organ failure syndrome (Pittirui et al), 378

Limited effects of prostaglandin inhibitors in *Escherichia coli* sepsis (Hulton, Johnson, Wilmore), 291

Septic endarteritis after percutaneous transluminal angioplasty (Krupski, Pogany, Effeney), 359

**Septicemia**

Released granulocytic elastase: Indicator of pathobiochemical alterations in septicemia after abdominal surgery (Duswald et al), 892

**Serotonin**

Effects of serotonin on canine bile formation (Kortz et al), 907

**Serum suppressor factor**

Cellular immune defects in patients with melanoma involving interleukin-2-activated lymphocyte cytotoxicity and serum suppressor factor (Balch, Itoh, Tilden), 151

**Shock**

Control of gastric vascular resistance in cardiogenic shock (Bulkley et al), 213

**Shock, contd.**

Extracellular-intracellular lactate gradients in skeletal muscle during hemorrhagic shock in rat (Pearce, Connett, Drucker), 625

Role of lung innervation in hemodynamic response to hypertonic sodium chloride solutions in hemorrhagic shock (Younes et al), 900

**Shunt**

Budd-Chiari syndrome with obstruction of inferior caval vein: Successful treatment by cavosplenoatrial shunt (Segadal et al), 63

Modified distal splenorenal shunt with expanded polytetrafluoroethylene interposition (Nagasue et al), 870

Portacaval shunt in treatment of primary Budd-Chiari syndrome (Pezzouli et al), 319

**Skin**

Further studies of putative cross-linking amino acid (3-deoxy-pyridinoline) in skin from patients with abdominal aortic aneurysms (Tilson), 888

Incidence of wound infection after stapled or sutured bowel anastomosis and stapled or sutured skin closure in humans and guinea pigs (Panton et al), 20

**Sodium chloride**

Role of lung innervation in hemodynamic response to hypertonic sodium chloride solutions in hemorrhagic shock (Younes et al), 900

**Solution, cardioplegic**

Functional endothelial damage by high-potassium cardioplegic solutions to saphenous vein bypass grafts (De Caterina et al), 465

**Somatostatin**

Presence of neuron-specific enolase and somatostatin in human parathyroid tissues (Weber et al), 1008

**Sphincter**

Assessment of an implantable ileostomy sphincter (Chandler et al), 72

**Stenosis**

Intestinal atresia and stenosis: Analysis of survival in 120 cases (Rescorla and Grosfeld), 668

Prostacyclin production in regions of arterial stenosis (Qvarfordt et al), 484

**Stomach**

Comparison of gastric mucosal blood flow as measured by H<sub>2</sub> gas clearance and microspheres during secretory stimulation and inhibition (Soybel et al), 174

Control of gastric vascular resistance in cardiogenic shock (Bulkley et al), 213

Effect of 16,16-dimethyl prostaglandin E<sub>2</sub> on gastric epithelial cell membrane potentials and resistances (Ashley, Soybel, Cheung), 166

Opposing central and peripheral actions of brain-gut peptides: Basis for regulation of gastric function (Pappas, Taché, Debas), 183

**Substance P**

Verapamil inhibition of intestinal effects of substance P (Zinner et al), 230

**Survival**

Intestinal atresia and stenosis: Analysis of survival in 120 cases (Rescorla and Grosfeld), 668

**Suture**

Bacterial colonization of percutaneous sutures (Gristina et al), 12

**Sympathectomy**

Surgical sympathectomy increases pancreatic polypeptide response to food (Larson, Sullivan, O'Dorisio), 236

**Synthesis**

Increases in gallbladder prostaglandin synthesis before formation of cholesterol gallstones (LaMorte et al), 445

Protein synthesis in tumor-influenced hepatocyte (Warren, Jeevanandam, Brennan), 275

**T****Tachycardia**

Histopathologic factors conducive to experimental ventricular tachycardia (Wetstein et al), 532

**Technetium/thallium**

Computerized technetium/thallium scans and parathyroid reoperation (Skibber et al), 1077

**Testicle**

Morphologic and functional studies of rat hypercalcemia-associated testicular tumor maintained in cell culture (Saxe et al), 1013

**Thallium/technetium**

Localization of residual medullary thyroid cancer by thallium/technetium scintigraphy (Talpos et al), 1189

**T helper cell**

Defective antigen presentation to a cloned T helper cell by macrophages from burned mice can be restored with interleukin-1 (Kupper et al), 199

**Thermal injury**

Spontaneous lymphocyte activity: Important but neglected component of immunologic profile of thermally injured patient (Deitch), 587

**Thrombosis**

Carotid artery thrombosis associated with lupus anticoagulant (Baker et al), 612

Indium 111 platelet imaging for detection of deep venous thrombosis and pulmonary embolism in patients without symptoms after surgery (Clarke-Pearson et al), 98

**Thromboxane A<sub>2</sub>**

Prostacyclin and thromboxane A<sub>2</sub> moderate postischemic renal failure (Lelcuk et al), 207

**Thyroid**

Betablockers compared with antithyroid drugs as preoperative treatment in hyperthyroidism: Drug tolerance, complications, and postoperative thyroid function (Lennquist et al), 1141

Declining role of frozen section in surgical planning for thyroid nodules (Hamburger and Hamburger), 307

Epidermal growth factor receptors in normal and neoplastic thyroid tissue (Duh et al), 1000

Further evidence of validity of risk group definition in differentiated thyroid carcinoma (Cady et al), 1171

Hürthle cell tumors of thyroid gland (Heppe et al), 1162

Incidence of cancer in surgically treated thyroid nodules based on method of selection (Lo Gerfo et al), 1197

Incidence of thyroid carcinoma in patients with Hashimoto's thyroiditis and solitary cold nodules (Ott et al), 1202

Localization of residual medullary thyroid cancer by thallium/technetium scintigraphy (Talpos et al), 1189

Malignant lymphoma of thyroid gland: Clinical and pathologic study of twenty cases (Rasbach et al), 1166

Management implications from routine needle biopsy of hyperfunctioning thyroid nodules (Walfish, Strawbridge, Rosen), 1179

Postoperative thyroid storm after lithium preparation (Reed and Bradley), 983

Pregnancy and surgical thyroid disease (Rosen, Walfish, Nikore), 1135

Thyroid carcinoma in Graves' disease (Farbota et al), 1148

Transsternal operations in thyroid cancer (Niederle, Roka, Fritsch), 1154

**T-lymphocyte**

"Activated" T-lymphocyte levels in the spontaneously diabetic BB rat syndrome (Francfort et al), 251

**Tomography**

Use of computerized axial tomography versus peritoneal lavage in evaluation of blunt abdominal trauma: Prospective study (Davis et al), 845

**Transfusion**

Influence of ultraviolet irradiation on blood transfusion effect (Balshi, Francfort, Perloff), 243

Serial changes in primary hemostasis after massive transfusion (Harrigan et al), 836

**Transplant**

Auxiliary transplantation of part of liver improves survival and provides metabolic support in pigs with acute liver failure (Reuvers et al), 914

De novo hemolytic uremic syndrome in renal transplant recipients immunosuppressed with cyclosporine (Van Buren et al), 54

General surgical complications in heart and heart-lung transplantation (Steed et al), 739

**Transplant, contd.**

Improved results of multiple renal transplantation in children (So et al), 729

Mismatched living, related donor renal transplantation: Prospective, randomized study (Sommer and Ferguson), 267

**Trauma**

Traumatic injury to proximal superior mesenteric vessels (Sirinek and Levine), 831

Use of computerized axial tomography versus peritoneal lavage in evaluation of blunt abdominal trauma: Prospective study (Davis et al), 845

**Tuberculosis**

Latent mammary tuberculosis: Case report (Wapnir et al), 976

**Tumor**

Carcinoid tumors of gastrointestinal tract: Presentation, management, and prognosis (Thompson et al), 1054

Hürthle cell tumor behavior: Dilemma and resolution (Rosen, Luk, Katz), 777

Hürthle cell tumors of thyroid gland (Heppe et al), 1162

Monoclonal antibodies in human renal cell carcinoma and their use in radioimmune localization and therapy of tumor xenografts (Lange et al), 143

Morphologic and functional studies of rat hypercalcemia-associated testicular tumor maintained in cell culture (Saxe et al), 1013

Partial hepatectomy on cirrhotic liver with right lateral tumor (Lee, Chao, Lin), 942

Protein synthesis in tumor-influenced hepatocyte (Warren, Jeevanandam, Brennan), 275

Spectrum of catecholamine-secreting tumors of organ of Zuckerkandl (Altergott et al), 1121

**Tyrosine**

Increased dependence of leucine in posttraumatic sepsis: Leucine/tyrosine clearance ratio as an indicator of hepatic impairment in septic multiple organ failure syndrome (Pittiruti et al), 378

**U**

**Ulcer**

Varicose ulcer of upper extremity (Davis et al), 616

**Urinary diversion**

Pathophysiology of hyperchloremic metabolic acidosis after urinary diversion through intestinal segments (Koch and McDougal), 561

**V**

**Vagotomy**

Gallbladder filling and response to cholecystokinin are not affected by vagotomy (Pellegrini et al), 452

**Varicosity**

Varicose ulcer of upper extremity (Davis et al), 616

**Vein**

Budd-Chiari syndrome with obstruction of inferior caval vein: Successful treatment by cavosplenoatrial shunt (Segadal et al), 63

**Velocity**

Low flow velocity predicts failure of femoropopliteal and femorotibial bypass grafts (Bandyk, Cato, Towne), 799

**Vena cava**

New approach to wounds of aortic bifurcation and inferior vena cava (Salam and Stewart), 105

**Ventricular septal defect**

Hemodynamic effects of surface cooling-induced hypothermia on immature pigs with ventricular septal defects (Ganzel et al), 516

**Verapamil**

Verapamil inhibition of intestinal effects of substance P (Zinner et al), 230

**Vessel**

Traumatic injury to proximal superior mesenteric vessels (Sirinek and Levine), 831

**Vibrio**

Necrotizing soft-tissue infections caused by marine vibrios (Howard et al), 126

**Vitamin A**

Vitamin A and retinoic acid: Induced fibroblast differentiation in vitro (Demetriou et al), 931

- W**
- Wound**  
Decreased wound neutrophils and indiscrete margination in pathogenesis of wound infection (Yurt and Pruitt), 191  
Herpes simplex burn wound infections: Epidemiology of case cluster and responses to acyclovir therapy (Brandt et al), 338  
Incidence of wound infection after stapled or sutured bowel anastomosis and stapled or sutured skin closure in humans and guinea pigs (Panton et al), 20  
New approach to wounds of aortic bifurcation and inferior vena cava (Salam and Stewart), 105  
Serious wound infections in burned patients (Kagan et al), 640  
Which cephalosporin for wound prophylaxis? Experimental comparison of three drugs (Scher and Jones), 30
- X**
- Xenograft**  
Monoclonal antibodies in human renal cell carcinoma and their use in radioimmune localization and therapy of tumor xenografts (Lange et al), 143
- Z**
- Zuckerkindl**  
Spectrum of catecholamine-secreting tumors of organ of Zuckerkindl (Altergott et al), 1121