Investigation of Serum Protein Patterns in Patients Undergoing Operation. (20760)

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(Introduced by C. L. Gemmill.)

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The disturbance of protein equilibrium following injury has been extensively studied in humans as well as in experimental animals(1,2). A negative nitrogen balance occurring as the first phase of the changed metabolism, and designated by Browne *et al.* (3) as the catabolic phase, is followed during the convalescence by an anabolic phase characterized by a positive nitrogen balance. The changes in the serum protein pattern of 6 patients following injury or surgery has been investigated by Cuthbertson and Tompsett (4). They found a decreased albumin and increased globulin fraction following injury. The serum components were separated by neutral salt precipitation. By this method the α_1 and α_2 globulins are not separated from the albumin.

The recent development of paper strip electrophoresis(5) made possible the study of the changes of all protein components in small amounts of blood without causing any discomfort to the patients. The result of such an investigation on patients undergoing surgery is presented.

Procedures and method. The sera of 45 patients were investigated. The patients were grouped in 3 main categories: patients with tuberculosis undergoing chest surgery, patients with uro-genital trouble, mostly benign prostatic hypertrophy, and patients with gastrointestinal and pulmonary cancer. Both sexes of the white and Negro races were represented. The ages of the patients were between 17 and 84 years. Venous blood was collected 8 to 12 hours before operation, then at intervals 4 to 96 hours after operation, and up to 12 days when the patient was available. On each serum the total protein concentration was determined by a modified Linderstrøm-Lang specific gravity gradient(6). On most of the patients the hematocrit was determined pre- and post-operatively by the Wintrobe method. The separation of the protein components was carried out by paper strip electrophoresis in a tent type Durrum apparatus at room temperature. Strips of Whatman 3 MM filter paper 2.5 cm x 38 cm were used with veronal buffer of pH 8.55 and μ 0.05. A current of 2-2.5 ma per strip was applied for 7 hours at which time the albumin had migrated 8-10 cm. The strips were dried for 10 minutes at 110°, stained with bromophenol blue (0.01% bromophenol blue, 5% acetic acid, and 5% mercuric chloride) for 6-12 hours, washed 3 times with 2% acetic acid 10 minutes each time, and once with 2% acetic acid containing 0.5% sodium acetate. They were then blotted and dried for 10 minutes at 110° and cut at positions of least staining between the bands. The color was eluted from the finely cut paper fragments with 6 ml of 0.01 N NaOH and the optical density read in a Coleman Universal Spectrophotometer at 590 mμ.

Experiments were carried out to establish the effect of the time of drying, of washing, and of staining on the reproducibility of color readings. The drying of the strips between 10 and 30 minutes at 110°, the washing of the strips 10 minutes to 24 hours, and the staining between 6 and 48 hours had no significant effect on the estimated proportions of the components. The elution of the color was complete in about 1½ hours, after this time the color values found were constant up to 24 hours. If the strips, however, were stored, stained and dried but not eluted, the color faded measurably after 4 days. The fading of the albumin was more rapid than that of globulins, which resulted in too low albumin values. By repeated estimations of serum patterns of different samples from the same individual, differences of ±4% in the albumin, and differences of ±2% in the other serum components was found statistically sig-

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TABLE I. Serum Protein Patterns of Clinically Healthy Persons and Patients Prior to Operation.

Diagnosis	No. of cases	Total protein, g/100 ml Mean	Avg	Albumin* Mean	Avg	Alpha$_1$* Mean	Avg	Alpha$_2$* Mean	Avg	Beta* Mean	Avg	Gamma* Mean	Avg
Healthy	20	8.1	7.35	68.0	58.7	8.6	5.9	16.2	9.5	18.2	10.7	21.6	14.4
		7.1		47.8		4.1		4.1		7.5		10.4	
T.B.	24	8.3	7.0	65.6	54.5	7.0	5.9	15.5	11.0	15.8	11.7	24.4	17.4
		6.4		41.5		3.9		8.1		6.4		9.9	
Ca	7	9.9	7.8	53.5	46.7	8.1	6.8	16.1	13.3	18.7	13.5	31.6	18.4
		6.2		31.8		5.4		11.3		11.1		9.4	
Bg. prostatic obst.	8	8.5	6.8	53.2	48.6	9.0	7.7	19.8	14.1	15.7	13.1	19.9	16.9
		6.9		40.0		5.9		9.3		11.4		12.9	
Misc.	6	8.4	7.1	58.0	48.0	8.4	6.1	13.8	10.8	13.4	12.8	33.7	22.0
		6.9		31.9		4.6		8.3		11.8		16.2	

* Values are % of total protein.

TABLE II. Serum Protein Patterns in Patients Showing High Gamma Globulin.

Diagnosis	Total protein, g/100 ml	Albumin*	Alpha$_1$*	Alpha$_2$*	Beta*	Gamma*
Ca of kidney	7.6	44.7	4.8	9.6	13.4	27.5
Bronchiectasis	8.4	31.9	7.2	13.8	13.3	33.8
Pulmonary T.B.	7.7	47.8	3.9	8.5	9.7	30.1
Pulmonary Ca	8.1	31.8	8.1	15.1	12.7	31.3
Empyema	7.4	40.0	7.0	11.0	10.0	32.0
Pulmonary T.B.	8.7	38.5	6.2	10.5	10.0	34.8

* Values are % of total protein.

nificant ($P > 0.05$). In evaluating the post-operative sera these figures were taken as a basis for designating changes.

Results. No differences were found in the serum proteins with regard to the sex, race, or age of the patient. The type of narcosis used did not seem to influence the serum protein pattern. The preoperative sera of patients were compared with sera from clinically healthy persons (Table I). The total protein concentration was about the same in all groups. In the preoperative groups the albumin tended to be lower and the globulins higher than in the healthy controls. This difference was least pronounced in cases of patients with tuberculosis. All these patients were treated preoperatively for a prolonged time in tuberculosis sanatoriums where they received a high protein high caloric diet. The mobilities of the components in the pre-operative sera did not differ from the healthy persons except in 3 cases in which an additional α_2 (2 cases) and an additional beta globulin (one case) was found. Increased gamma globulin, higher than 25% was found in 6 cases (Table II). Five of these had advanced pulmonary disease.

The protein patterns in healthy persons did not vary significantly over many days (Table III). Changes found in the serum patterns of patients undergoing surgery are shown in Fig. 1 and Table IV. Immediately after operation the albumin dropped in one-third of the cases, and the α_1 and α_2 globulins

TABLE III. Variations in Composition of Serum of Clinically Healthy Persons.

	Total protein, g/100 ml	Albumin*	Alpha$_1$*	Alpha$_2$*	Beta*	Gamma*
1	7.1	67.1	4.7	7.4	9.5	11.3
	7.9	64.4	5.9	9.1	10.0	11.6
	7.1	67.5	6.0	7.6	8.1	10.8
2	7.1	59.1	6.4	10.5	10.9	13.1
	7.1	60.0	5.8	11.1	12.6	10.5
	7.1	56.5	5.9	12.4	12.8	12.4
3	7.3	61.5	5.4	9.2	8.6	15.3
	7.5	59.5	6.1	8.8	8.6	17.0
	7.5	64.2	5.0	9.0	8.3	13.5
	7.3	63.0	5.7	8.5	9.1	13.7
	7.5	65.0	4.4	6.6	8.5	15.5
	7.7	62.5	5.0	6.5	10.0	16.0
	7.5	64.2	3.7	7.8	9.1	15.2
4	7.6	47.8	6.9	10.5	11.9	22.9
	7.5	49.0	6.3	10.6	12.4	21.7
	6.1	51.0	6.0	10.1	11.5	21.4

* Values are % of total protein.

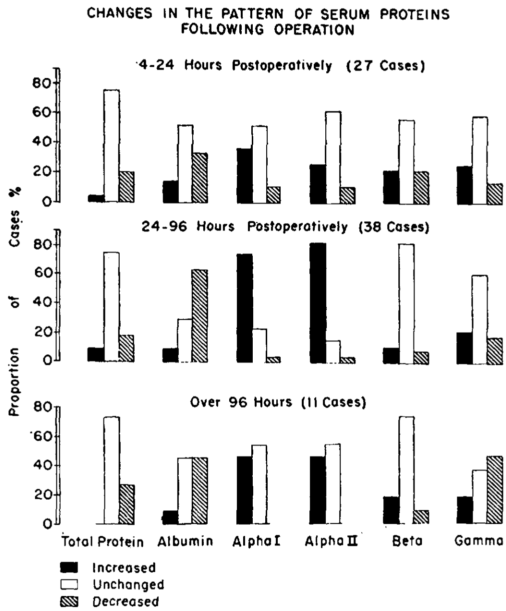

FIG. 1

rose. In the following 3 days in 68% of all cases the albumin was significantly decreased, the α_1 and α_2 globulins were increased in 76% and 82% of the cases, respectively. The beta globulins remained unchanged in 82% and the gamma globulin in 61% of all cases. The mean of the total proteins did not change more than by 1 g/100 ml during the time of observation. The hematocrit in the preoperative patients was generally slightly below normal (mean 42% for both sexes), it increased slightly in the first 24 hours postoperatively (mean 45%), and it decreased during the following 4 days (mean 40.8%). No significant difference was found in the post-operative response in the groups of patients.

The components of serum proteins did not show differences in their mobilities before and after operation with the exception of 3 cases in which the samples taken after operation showed components of different mobilities. As all the patients received blood, saline, or glucose in quantities of 500 to 2500 ml during and immediately after the operation, the absence of changes in the total proteins or protein patterns was remarkable.

Discussion. When evaluating the changes in the serum protein patterns following surgical operations it should be borne in mind that the preoperative levels of the components in this series and presumably in all other similar investigations have already shifted in a direction of the levels found in the postoperative period. The decreased albumin together with an increased globulin level prior to operation might be ascribed to protein depletion of the patients due to their respective diseases. It has been observed that a negative nitrogen balance does not occur after injury in patients who have very little nitrogen reserve due to undernourishment or protein depletion. The gradual decrease of the serum albumin in the first postoperative days is in agreement with the findings of Cuthbertson(7) and of Grossman *et al.*(8) that the nitrogen excretion in urine was not great immediately after operation and that only after the lapse of 3 to 4 days did it become considerable.

TABLE IV. Changes in Serum Albumin, Alpha$_1$, and Alpha$_2$ Globulins Following Surgery.

Time	No. of cases	Albumin* Range	Albumin* Mean	Alpha$_1$* Range	Alpha$_1$* Mean	Alpha$_2$* Range	Alpha$_2$* Mean
Pre-op.	45	68.0 47.8	51.2	8.6 4.1	6.3	16.2 4.1	11.4
Post-op.							
4-24 hr	27	55.1 45.0	46.9 (9)†	14.0 7.9	9.1 (10)	21.0 12.4	14.5 (7)
24-96 "	38	50.8 21.8	41.5 (24)	12.6 8.1	9.6 (28)	19.2 12.3	15.1 (31)
96+ "	11	49.0 38.4	44.8 (5)	10.7 7.4	9.9 (5)	20.0 16.0	16.4 (5)

* Values are % of total proteins.

† Figures in parentheses represent No. of sera in these groups in which changes were statistically significant, *i.e.*, greater than 4 for albumin or 2 for the globulins.

The mechanism and biological significance of the negative nitrogen balance after injury is not clear. It is generally assumed that the direct destruction of tissue during operation is not the responsible factor. Hormonal and enzymatic factors might be decisive. There are many similarities in the nitrogen metabolism in response to injury and the general "alarm" or "adaptation" syndrome(9). The adrenocortical hormone of the pituitary(10) and the 11-oxycorticoid(11) produced negative nitrogen balance. Apart from the decrease of the total mass of liver proteins, the concentration of protein enzymes(12,13) including D-amino acid oxidase(14) was found to be decreased in the liver of rats fed a low protein diet. A disturbance of the enzyme systems of the liver, especially those connected with the protein metabolism, may be at least in part responsible for the postoperative protein disequilibrium.

Summary. 1. Distribution of serum protein components was investigated by the use of paper strip electrophoresis in 45 patients undergoing surgery. 2. A significant decrease in the albumin and increase of α_1 and α_2 globulins occurred in one-third of the cases during the first 24 hours following operation. These changes were present in 80% of all cases during the following 4 days. Thereafter the serum components tended to return to their preoperative levels. Components with mobilities different from those found preoperatively occurred in 3 cases after operation. 3. Total serum proteins and the hematocrits did not show any significant variation in the early postoperative period.

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An Electrophoretic Method for Titration of Antisera and Its Application to Anti-Tumor Sera.* (20761)

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Coulter(1) reported that the electrophoretic mobility of washed sheep erythrocytes which had been sensitized with homologous antiserum was less than that of normal washed cells. Shortly thereafter De Kruif and Northrop(2) concluded from their observations on the behavior of *Bacillus typhosus* in the presence of its homologous antiserum that the antibody combines with the surface of the bacteria, producing a surface charge density dependent upon the proportion of the surface so combined. This specific effect of homologous antiserum has since been demonstrated for the

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