much less absorbed by the Raney nickel than the free peptides, and treatment of the reaction mixture is reduced to only its filtration and evaporation, the product being subjectable to mass spectrometry without purification. The α-aminobutyric acid residue behaves similarly to other aliphatic amino acids[1] under electron impact and its position in the peptide chain (and consequently that of the methionine residue) can readily be determined. If the peptide contains an alanine residue as well as cysteine (cystine) the Ni/Al alloy used for preparing the catalyst should be leached in D_2O so that the cysteine (cystine) residue is converted into deuteroalanine residue.

Figure 1 shows as example the mass spectrum of Dec-Met-Phe-Gly-Cys(CH_2COOMe)-OMe (1) which is very complicated and moreover difficult to reproduce owing to thermal decomposition of this substance during mass spectrometry. On the contrary, the mass spectrum of Dec-AmBut-Phe-Gly-Ala-OMe (2) prepared by treatment compound (1) with Raney nickel as described above, is simple and quite readily deciphered. It is also noteworthy that the temperature of mass spectrometry of this substance is 100 °C below that required for compound (1).

Hence desulfurization considerably simplifies the mass spectrometric determination of the amino acid sequence of the sulfur-containing peptides and at the same time extends the limits of this method.

Выводы. Показано, что десульфурирование эфиров серусодержащих N-ацилпептидов никелем Ренея в диметилформамиде при 20° существенно упрощает определение аминокислотной последовательности масс-спектрометрическим методом и одновременно расширяет границы этого метода.

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Electrophoretic Behavior of Sonicated Human Serum Proteins

Although gaseous cavitation has been widely used to disrupt cells and subcellular particles[1], little is known about its influence on serum or plasma proteins. Preliminary studies[2] indicate such forces render fibrinogen molecules incapable of forming a fibrin clot and solubilize lipoproteins through the uptake of additional protein[3].

Qualitative or quantitative details about changes in serum or plasma proteins mediated by gaseous cavitation are still lacking. Therefore, attempts were made to characterize the phenomenon by investigating sonic effects on the electrophoretic behavior of proteins in normal and abnormal sera as well as purified preparations of human serum albumin, γ-globulin and β-lipoprotein.

Materials and methods. Sera from normal subjects and patients with various diseases were transferred in 2.5 ml aliquots to heat-resistant glass tubes placed in an ice-water bath. A titanium probe (0.75 inches in diameter, end ratio 3.6:1) was immersed 1–2 mm below the surface of the serum and sound frequencies of approximately 20 kc/sec were generated with an MSE disintegrator[4] for 10 or 20 min. Samples remained several degrees below room temperature during sonic oscillation.

Serum before and after exposure to ultrasound were analyzed during the Micro Zone[5] electrophoretic system. Electrophoretic distributions on cellulose acetate were determined densitometrically after staining the strips with Ponceau S. Measurements of the total protein were made in terms of the biuret color reaction.

Results and discussion. A series of 12 pools, each containing 3 different sera, were prepared from 36 healthy individuals. Each pool was subjected to electrophoresis before as well as after 10 and 20 min exposure to ultrasound. The qualitative and quantitative changes induced by sonication were similar in all samples. After 10 min of oscillation, the protein content of the α- and β-globulin zones increased (Figure 1). This change, together with a reduction in albumin and γ-globulin fractions, was more conspicuous following 20 min of ultrasound.

In a typical example, protein in the albumin zone declined from an initial value of 4.6–3.5 g/100 ml after the final period of oscillation (Table). This was accompanied by a reduction in the γ-globulin fraction to about $^1/_3$ its original value. The protein displacement occurring with 20 min of oscillation caused a decline in the ratio of albumin to globulin from 2.4 to only 1.7.

Saline solutions containing purified human serum albumin[6] (6.25 g/100 ml) and γ-globulin[6] (0.7 g/100 ml) as well as a mixture of the 2 proteins at the stated concentrations were subjected to electrophoresis before and after sonication for 10 min. Such treatment of the albumin solution caused about 16% of the protein to migrate less rapidly anodally (Figure 2). When the γ-globulin solution was treated similarly, more than 30% of the protein moved into the β-globulin zone (Figure 3). Oscillation of the γ-globulin-albumin mixture caused the appearance of only a single new position zone, residing in the β-globulin position (Figure 4). About $^1/_3$ of the total protein was displaced into this area, with little or no increase in protein-staining material in the α_1-position.

Sera collected from patients with various diseases were examined electrophoretically before and after 10 min of sonic radiation. The variability of the responses obtained is illustrated by the patterns secured with specimens from 2 subjects with multiple myeloma. Sera from these patients both showed a typical peak of myeloma protein in the γ-globulin area. Following 10 min exposure to ultrasound, the pattern from 1 patient indicated a marked displacement of protein into the β- and α_2-globulin zone,

[1] D. E. HUGHES and W. L. NYBORG, Science *138*, 108 (1962).
[2] R. L. SEARCY, L. M. BERGQUIST, N. M. SIMMS, D. JOHNSTON and J. A. FOREMAN, Nature *206*, 795 (1965).
[3] R. L. SEARCY and L. M. BERGQUIST, Biochim. biophys. Acta *106*, 603 (1965).
[4] Instrumentation Associates Inc., New York (USA).
[5] Spinco Division, Beckman Instruments, Palo Alto (California, USA).
[6] Hyland Laboratories, Los Angeles (California, USA).

Ultrasonically induced alterations in the electrophoretic distribution of protein in normal human serum

Oscillation time (min)	Protein levels Albumin %	g/100 ml	α_1-globulin %	g/100 ml	α_2-globulin %	g/100 ml	β-globulin %	g/100 ml	γ-globulin %	g/100 ml
0	71.7	4.6	0.77	0.05	7.4	0.48	10.6	0.68	10.2	0.66
10	63.3	4.1	3.1	0.2	17.6	1.14	10.2	0.66	5.5	0.35
20	54.9	3.5			41.9	2.8			3.1	0.20

the material in this area almost tripling. Similar treatment of the sample from the other patient failed to produce such a pronounced electrophoretic change. In this case, all globulin fractions remained discrete, although the protein content of the α_1-, α_2-, and β-globulin zones were demonstrably increased while that in the albumin and γ-globulin regions were diminished.

Oscillation of serum from an individual with pyelonephritis and hypergammaglobulinemia for 10 min produced an impressive shift of protein material into the α_2- and β-globulin area. In this instance, however, the electrophoretic behavior of albumin was virtually unaffected by sonication.

Serum obtained from a subject during a severe episode of diabetic acidosis was highly lactescent. Treatment with ultrasound lessened the lactescence and caused almost half of the protein to occupy the α_2- and β-globulin region. The sonically induced reduction in protein in the γ-globulin zone was much more striking than was that in the albumin region.

Ultrasound causes a reproducible change in the electrophoretic behavior of normal human serum consisting of an increase in material migrating in the α- and β-globulin zones with a reduction in the albumin and γ-globulin fractions. Examination of purified human serum albumin and γ-globulin before and after sonication indicates that a proportion of these proteins is altered in a manner affecting their electrophoretic mobility. Surprisingly, only one, rather than two, anomalous protein component is distinguished electrophoretically in oscillated serum

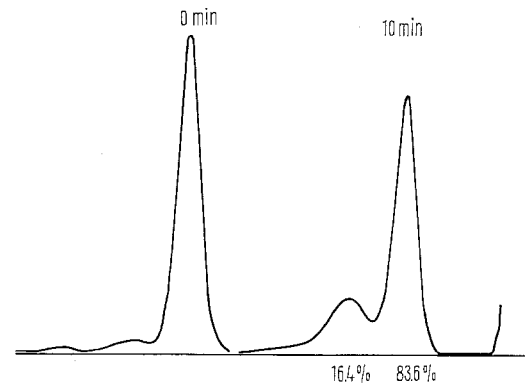

Fig. 2. The electrophoretic behavior of purified human serum albumin before and after 10 min exposure to ultrasound.

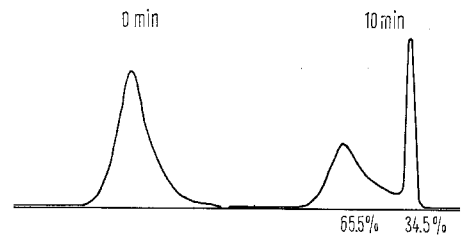

Fig. 3. The electrophoretic behavior of purified human serum γ-globulin before and after 10 min exposure to ultrasound.

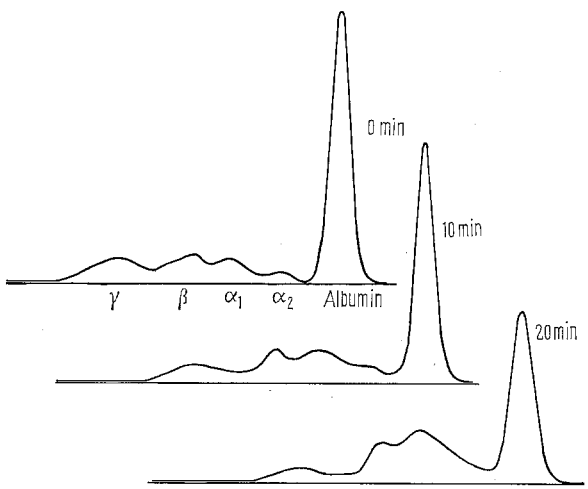

Fig. 1. Electrophoretic patterns of normal serum before and after 2 timed exposures to ultrasonic frequencies.

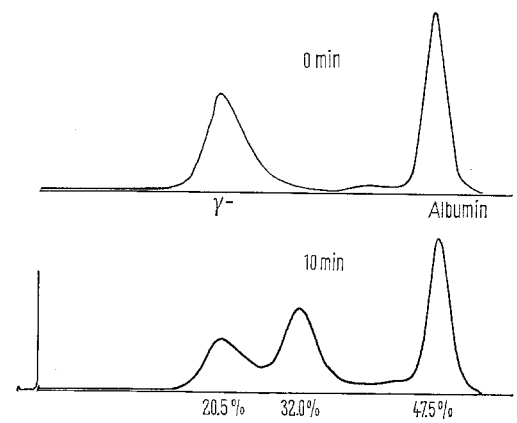

Fig. 4. The electrophoretic behavior of a human serum albumin-γ-globulin mixture before and after 10 min exposure to ultrasound.

albumin-γ-globulin mixtures. This unusual protein fraction electrophoretically resembles the peak formed when γ-globulin alone is sonicated.

The electrophoretic data and other evidence suggest that ultrasound causes the formation of an albumin-globulin complex. Moreover, while the complex retains certain solubility properties of γ-globulin, those of albumin seem obscured. The total protein content of salt precipitable γ-globulin fraction has been shown to increase after whole serum is sonicated[3]. The sonically formed complex probably contains an outer layer of γ-globulin surrounding the albumin molecules. Hence, only a single new fraction residing in the β-globulin region can be distinguished electrophoretically after exposure of albumin-γ-globulin mixtures to ultrasound.

The atypical electrophoretic patterns observed in some diseases probably denote the presence of unusual proteins. Changes in electrophoretic behavior following sonic treatment likely depends upon the quantitative and qualitative nature of the proteins in serum. Ultrasound might be useful for detecting the presence of certain abnormal serum proteins.

Zusammenfassung. Normale und abnorme Seren sowie gereinigtes menschliches Serumalbumin und Serumglobulin, wurden vor und nach Ultraschalleinwirkung auf elektrophoretisches Verhalten untersucht. Die Unterlagen deuten auf eine Bildung aussergewöhnlicher Komplexe zwischen gewissen Serumproteinen, wahrscheinlich γ-Globulin und Albuminfraktionen, durch ultrasonische Frequenzen hin.

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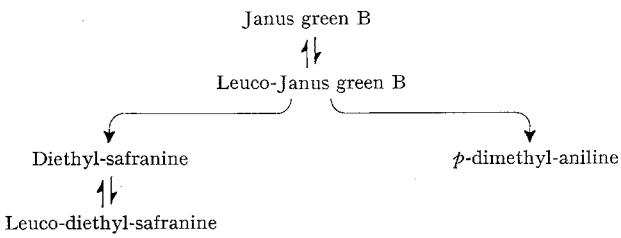
Janus Green B and Experimental Syndactyly in Chick Embryos

Very small amounts of Janus green B (JB) injected into the amniotic fluid of incubated eggs (in the 29 Hamburger-Hamilton (HH) stage) lead to syndactyly in all survivors[1,2].

According to HAVEMANN[3], JB (diethyl-safranine-azo-*p*-dimethylaminobenzene) can be reversibly reduced to leuco-Janus green B, a hypothetical intermediary compound, which on further reduction splits by an irreversible process into pink diethyl-safranine (DES) and *p*-dimethyl-aniline (DMA). This is the final reduction stage in the living cell, while a leuco-DES, which can be obtained by catalytical hydrogenation, is instantly oxidized into pink DES by O_2.



As JB electively taints in green living mitochondria, an interference of the dye with biological oxidation has been assumed[4]. We actually found an interaction between JB and riboflavine, the active site of FAD: (1) JB reacts with dihydroriboflavine and forms a water-insoluble greenish compound. No interaction between JB and riboflavine could be observed in the visible range of the spectrum. (2) The reduction of JB with ascorbic acid is accelerated by small amounts of riboflavine. (3) The polarographic reduction wave of riboflavine shifts to more positive values on the addition of JB. In Figure 1 the molar ratio of the compounds is presented.

In an experimental model, we observed that very small amounts of JB exert stimulation on yeast oxygen uptake, as determined by polarography in paraffin-oil sealed media. Glucose has been added to yeast suspensions in 7.2 pH phosphate buffer (Dulbecco isotonic medium for tissue cultures), until further addition of glucose did not increase the rate of oxygen uptake. Then, progressive amounts of JB were added, increasing respiration rate up to 45% over the maximal level attained by saturation with glucose.

The same experiment was repeated with acetaldehyde as food instead of glucose, for starved yeast. The results obtained were most similar to those with glucose (Figure 2). As acetaldehyde is directly oxidized by the respiratory chain[5], this similitude suggests an action of the dye upon this common metabolic pathway.

As long as oxygen concentration could be maintained by constant air bubbling, above approximately 50% of saturation, JB was not reduced by the yeast and the respiration stimulus persisted. In lack of aeration, O_2 concentration of the medium rapidly fell and JB was reduced to pink DES, while oxygen uptake sank to very low values, assumedly by toxic action. JB rapidly turns into pink DES in the allantoic fluid of the chick embryos, so it had to be investigated whether the dye itself or some of its reduction products is actually responsible for the syndactylism induced.

A comparative testing of JB and the equivalent amounts of the reduction compounds (DES + DMA) proved that only the dye itself is teratogenetical. However, a toxic effect of the reduction products, as concluded from the high lethality, can be admitted.

It can be assumed that JB has an action upon the respiratory chain (at least in yeast) forming a FAD-JB type complex. The respiration stimulus observed suggests an electronic shunt role of this complex. METZNER[6], in

[1] B. MENKES and M. DELEANU, Revue roum. Embryol. cyt. Embryol. *1*, 1.65 (1964).
[2] M. DELEANU, Revue roum. Embryol. cyt. Embryol. *2*, 1.45 (1965).
[3] R. HAVEMANN, H. PIETSCH and H. WIELGOSCH, Z. wiss. Photogr. *59*, 100 (1960).
[4] Z. BRAUN, M. ERDÉLYI, Z. HARMATH, Acta morph. hung. *8*, 4 (1959).
[5] P. K. MAITRA and R. W. ESTABROOK, Arch. Bioch. Biophys. *121*, 117 (1967).
[6] H. METZNER, Hoppe-Seyler's Z. physiol. Chem. *349*, 1586 (1968).