

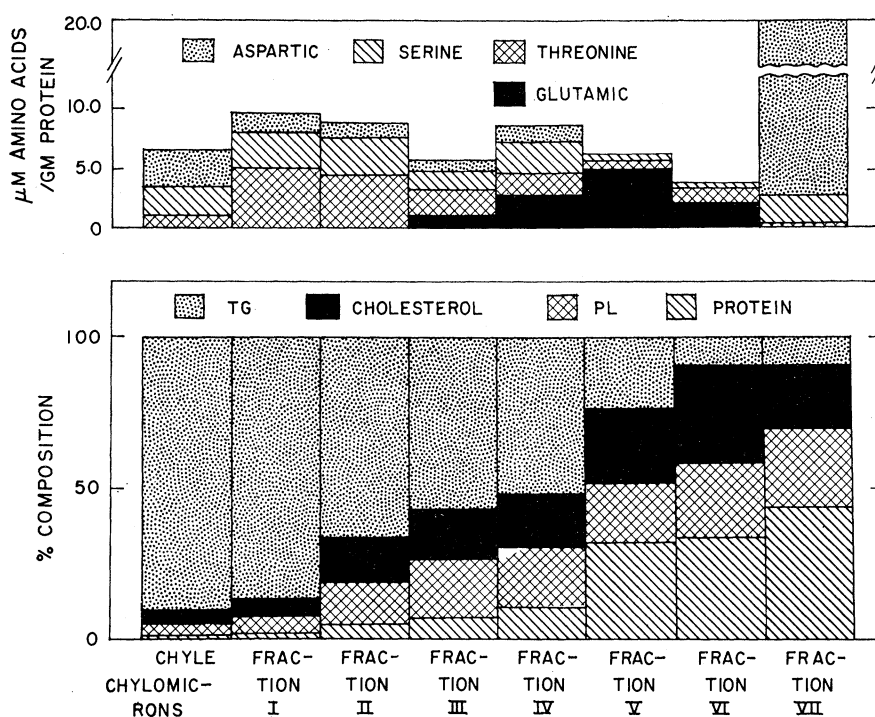


Fig. 1. N-Terminal amino acid and lipid composition of lipoproteins from chyle and plasma. Identical results were obtained from two patients in fraction III. The DNP aspartic acid recovery in fraction VII indicated a minimum molecular weight of 54,000. For dog plasma the same fraction contained only N-terminal aspartic acid with a recovery indicating a minimum molecular weight of 46,000 (13).

phate, pH 7.0, and recentrifuged. This process was repeated until constant protein concentration was achieved. After removal of fraction II, the soluble lipoproteins which floated were further washed to obtain fractions III and IV. The techniques of isolation of fractions V, VI and VII were essentially those described by Havel *et al.* (4) and represent lipoproteins that floated at densities 1.019, 1.063, and 1.21, respectively.

The lipid and protein content of each fraction was obtained according to the procedures used by Bragdon *et al.* (5) and was utilized in conjunction with data published by Oncley *et al.* (6) to approximate the range of S_f classes represented by each fraction. Fraction III corresponds roughly to S_f 40–100, fraction IV to S_f 20–50, fraction V to S_f 10–17, fraction VI to S_f 3–8, and fraction VII to the high density classes described by Gofman *et al.* (7).

The lipoproteins were freed of lipid by extraction with chloroform-methanol (2:1) and further extracted with alcohol, alcohol-acetone, and ether. The protein was reacted with dinitrofluorobenzene and then hydrolyzed. The DNP amino acids were chromatographed and measured quantitatively according to the procedure of Levy (8). The chromatographic procedure of Blackburn and Lowther (9) was used for final identification of the DNP amino acids. The cor-

rection factors used for losses during hydrolysis were those of Porter (10).

The results presented in Fig. 1 indicate that while N-terminal threonine and serine appear in all the fractions, they are the predominant N-terminal amino acids in fractions I to IV, the major carriers of triglyceride in plasma. N-Terminal aspartic acid appears in these fractions as well as in fraction VII, although the presence of N-terminal aspartic acid in all of these fractions, of course, does not necessarily mean that the proteins containing the aspartic acid are identical (11). N-Terminal glutamic acid, however, appears to be present only in fractions III to VI. It therefore is extremely unlikely that the chyle and plasma chylomicrons and the bulk of the other lipoproteins of density less than 1.006 are "convertible" to the major lipoprotein of D 1.006–1.063. Furthermore, the results indicate that the very low density lipoproteins, whose primary distinguishing feature is the high percentage of triglyceride, represent a group of proteins with N-terminal serine, threonine, and aspartic acid.

Since the proteins containing N-terminal serine, threonine, and aspartic acid do not appear to have any stoichiometric relationship to one another, it must be concluded that these proteins are not part of a molecular unit but are distinct entities. Obviously no functional role can

be assigned to any one of these proteins until they are isolated and studied separately although it would appear that one or more of them must be intimately associated with the transport of triglyceride.

Any concept concerning lipid transport in plasma must be based on a study of the metabolism of the individual proteins as well as the lipid moieties, since each protein may have a specific role in the metabolism of lipids. It is also apparent that the methods presently used for the isolation of plasma lipoproteins are not adequate for isolating homogeneous lipoproteins. New methods for their purification are now being developed in this laboratory to facilitate further studies of the metabolic and structural properties of plasma lipoproteins (12).

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References and Notes

- H. B. Jones *et al.*, *Am. J. Med.* 11, 358 (1951); F. T. Pierce, Jr., *Metabolism* 3, 142 (1954).
- J. Avigan, R. Redfield, D. Steinberg, *Biochim. et Biophys. Acta* 20, 557 (1956).
- B. Shore, *Arch. Biochem. Biophys.* 71, 1 (1957).
- R. J. Havel, H. A. Eder, J. H. Bragdon, *J. Clin. Invest.* 34, 1345 (1955).
- J. H. Bragdon, R. J. Havel, E. Boyle, *J. Lab. Clin. Med.* 48, 36 (1956).
- J. L. Oncley, K. W. Walton, D. G. Cornwell, *J. Am. Chem. Soc.* 79, 4666 (1957).
- T. W. Gofman *et al.*, *Plasma* 2, 413 (1954).
- A. L. Levy, *Nature* 174, 126 (1954).
- S. Blackburn and A. G. Lowther, *Biochem. J.* 48, 126 (1951).
- R. R. Porter, *Methods in Medical Research* 3, 256 (1950).
- The possibility exists that the N-terminal aspartic acid arises from albumin which has aspartic acid as the N-terminal amino acid. [H. van Vunakis and E. Brand, *Abstracts*, 119th meeting, *Am. Chem. Soc.*, (1951), p. 28c]. However, preliminary studies on dog chylomicron protein and the high density lipoproteins (fraction VII) indicate that N-terminal aspartic acid is, as in human beings, present in both fractions, and by use of a "fingerprinting" technique, both proteins were found to be identical.
- I express my appreciation to Dr. Donald S. Fredrickson for the procurement of the patients utilized in this study and to Dr. Edward Korn for his interest and stimulation in all phases of this work.
- M. Rodbell, unpublished results.

18 November 1957

Yellow Acid-Cation Complexes in Lake Water

Recent studies of the yellow materials present in natural waters and commonly called humins or tannins (1) have shown that these materials consist of ultraviolet fluorescing dicarboxylic hydroxy aliphatic organic acids of molecular weight approximately 450. They are probably unsaturated and are apparently non-nitrogenous, containing only carbon, hydrogen, and oxygen. These acids are present in

quantities that, for lakes, are remarkably large (at least 5 mg/lit in Linsley Pond, a small, but not highly colored, lake in Connecticut) and, aside from their coloring role and consequent effects on light absorption, are important in that they stimulate the growth of certain limnetic algae. In addition, they may be important chelating agents and, in fact, are capable of keeping iron in a nonprecipitable state at a pH as high as 9.5 for at least several weeks.

The problem of determining the number of yellow substances involved is not an easy one. For example, in Fig. 1, *A* is a photograph, made by ultraviolet light, of a paper chromatogram of an 80 percent ethanol extract of dried residue from the water of Zoar Lake, Connecticut; it shows results typical of those obtained from Connecticut lakes. (The solvent consisted of water, tertiary amyl alcohol, pyridine, and diethylamine, (25:25:25:1). The paper was Whatman No. 3.) Figure 1 *B* is a similarly run chromatogram of material from Panther Lake, Washington; it also shows regionally typical results. The two patterns consist of several zones and are very similar even though the lakes are separated by a distance of about 3000 miles.

Several possible explanations are apparent: (i) The chromatographic zones may represent a series of different acids, all of which are common to all of the lakes examined; (ii) the zones may represent dimers, trimers, or polymers of a small number (possibly one) of acids; (iii) the zones may represent different salts or complexes of one or more acids. On the basis of infrared spectra of eluted zones, and perhaps even on the basis of probability, the first possibility seems to be unlikely. The second may be true, especially in the light of the free hydroxyl groups, but is difficult to test. The third, or salt hypothesis, can be checked.

To test this hypothesis, advantage was taken of the fact that the materials, being acidic, are extractable from acidified lake water concentrates with ethyl acetate. Paper chromatograms of such extracts show two rather blurred zones of high R_f but otherwise have little detail (Fig. 1 *C*). However, if the preparation is mixed, before chromatography, with a solution of the salts of Ca, Mg, Na, K, and Fe, the story is quite different (Fig. 1 *D*). The two fast-running areas are present as before, but now the similarity to *A* and *B* is striking. Here, then, is a possible explanation for the chromatographic similarity of lakes, since the ions used are uniformly present.

Further experiments with single salts show that, while the iron apparently has no effect, the zone at the origin (extreme right in Fig. 1) is associated with magnesium and appears only in its pres-

ence. [On a previous occasion (1), Ca alone yielded a weak zone at the origin. This result is now believed to have been due to contamination with a magnesium salt.] The next zone (R_f about 0.10) appears only with Na, K, or Rb and not with Li. Flame photometric analyses of individually eluted zones yielded the same conclusion.

An interesting adjunct to these observations has come from a study of two lakes each of which lacked one of the characteristic chromatographic zones. One of these is a small pond near Olalla, British Columbia; a chromatogram of its waters is shown in Fig. 1 *E*. The zone at the origin is absent. When $MgCl_2$ (but not $CaCl_2$) is added to the ethanol extract before running, the zone appears (Fig. 1 *F*). The second lake, Hot Lake, is in Washington, and in this case the zone at the origin is strong but the second zone is vague or absent (Fig. 1, *G*). Addition to the ethanol extract before chromatography, of either NaCl or KCl restores this zone (Fig. 1 *H*). Thus, the earlier results linking certain zones with specific ions are confirmed.

It might therefore be expected that Olalla Pond would be deficient in Mg and Hot Lake low in Na and K. However, Olalla Pond contains, in addition to 30 mg of Na per liter, 7 mg of Mg per liter; and Hot Lake, which occupies the excavation of an old epsom salt mine and, in consequence, has about 14,000 mg of Mg per liter, also has 4500 mg of Na per liter and 640 mg of K per liter. This might suggest that the Na/Mg ratio is important in determining the chromatographic pattern, since Olalla Pond has a ratio of 4.3 compared to 0.32 for Hot Lake; but in spite of the encouraging fact that the two other Washington lakes that lack the "magnesium" zone—Soap Lake and Blue Lake—had ratios

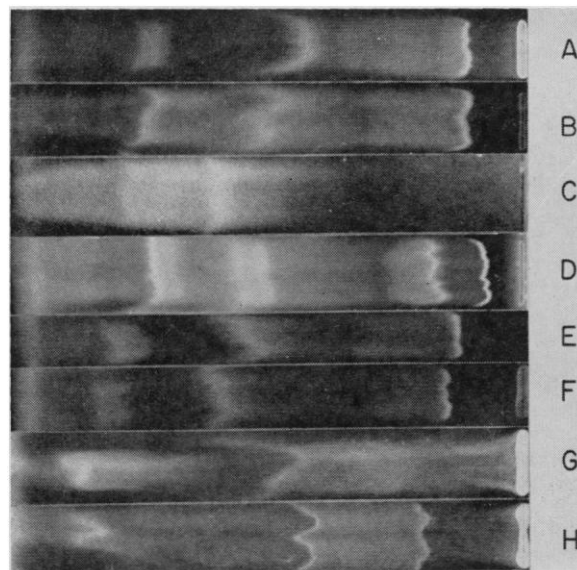
of 680 and 3.5 respectively, two more lakes had ratios of 5 and 10, respectively, and each had a strong zone at the origin. Thus, it was necessary to find another explanation.

With regard to the three lakes that lack the zone at the origin, the only unique factor was their high pH , which was above 8.4 in each case. (The pH of all of the other lakes studied was between 6.9 and 8.2.) That these three lakes still contained appreciable amounts of magnesium precluded the possibility that the absence of the "magnesium zone" was due to the precipitation of the metal as the hydroxide. Rather, it is more likely that the magnesium "salt" either does not form or that it dissociates at the higher pH , or that it becomes destroyed during evaporation, even though evaporation is carried out under vacuum.

To check on these possibilities, water from Vaseaux Lake, British Columbia, (pH 8.2, zone at origin present) was raised to pH 9.7 with Na_2CO_3 and, after filtering, was evaporated in the usual way and separated by chromatography. The zone at the origin was absent. A similar sample was acidified with HCl after it had been applied to the starting line on the paper. In this case the zone appeared, although it was reduced in intensity.

Similarly, a sample of water from Olalla Pond (pH 8.4, zone at origin absent), acidified just before running, showed a weak zone. Furthermore it was shown that the presence or absence of the zone is not due to its being reversibly decolorized and recolorized by alkali and acid, respectively. The second zone was in no way affected by these procedures. Thus, it seems as though the formation of the magnesium "salt" is favored by a low pH , or, as in the case of the addition

Fig. 1. Paper chromatograms of treated and untreated samples of lake water. Direction of solvent flow was from right to left. The appearance of the chromatograms results from their being photographed by ultraviolet light (see text for explanation of individual zones).



of $MgCl_2$ to water from Olalla Pond, by a high concentration of Mg. The reduction, as in Hot Lake, of the second zone or sodium "salt" in the presence of a very high concentration of magnesium is possibly due to competition for attachment sites by the ions.

Lakes, then, have a reasonably uniform complement of organic "salts" or perhaps "complexes," the uniformity resting largely on the inorganic ions common to all. High pH values or a very high concentration of a single ion may modify the pattern. That the yellow acids are intimately associated with the ionic metabolism of lakes is clear. In addition to the connection of these acids with the major ions, preliminary studies have shown possible involvement with such less abundant, but biologically important, ions as cobalt, iron, manganese, copper, and zinc.

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Reference

1. J. Shapiro, *Limnol. & Oceanog.* 2, 161 (1957). 8 November 1957

Effect of Iproniazid on Brain Levels of Norepinephrine and Serotonin

Iproniazid (1-isonicotinyl-2-isopropylhydrazine; Marsilid) was originally introduced as a drug for the treatment of tuberculosis, but was soon found to induce signs of central stimulation. Recent observations (1) indicate that the action on the central nervous system may be of value in treatment of depressed mental conditions.

Zeller *et al.* (2) were the first to ob-

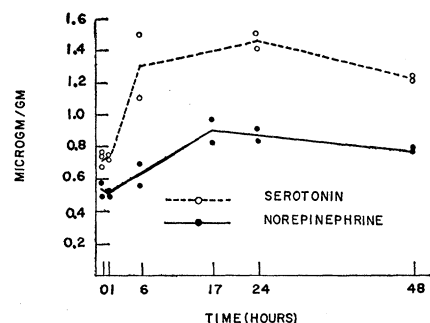


Fig. 1. Effect of a single large dose of iproniazid on serotonin and norepinephrine concentration in rabbit brain stem. Iproniazid (100 mg/kg) was injected subcutaneously. At various times thereafter animals were killed by intravenous injection of air, and the brain stems were analyzed. Each point represents the value from a single animal.

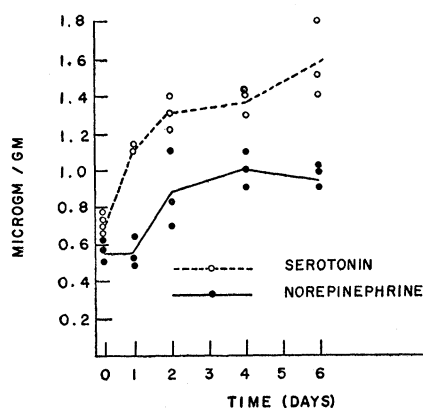


Fig. 2. Effect of daily doses of iproniazid on serotonin and norepinephrine concentration in rabbit brain stem. Iproniazid (25 mg/kg) was injected daily, subcutaneously. Animals were killed at various times by an intravenous injection of air, and the brain stems were analyzed. Each point represents the value from a single animal.

serve that iproniazid inhibits monoamine oxidase, an enzyme which can inactivate norepinephrine and serotonin, substances which may be involved in central regulatory mechanisms (3). In a recent paper (4) we have presented evidence that monoamine oxidase has a major role in the physiologic inactivation of both monoamines in the brain. The data presented in this report show that repeated doses of iproniazid induce a marked rise in the brain levels of both norepinephrine and serotonin, together with signs of central stimulation.

Iproniazid (100 mg/kg) was administered subcutaneously to rabbits, and the concentrations of norepinephrine and serotonin in the brain stem were measured at various times by previously described methods (5, 6). As is also reported by other workers (7, 8), the serotonin level increased markedly within a few hours after the administration of iproniazid. Norepinephrine levels also increased, but not as rapidly as those of serotonin (Fig. 1). No obvious pharmacological signs were evident.

Iproniazid was given daily in doses of 25 mg/kg subcutaneously to another group of rabbits. Serotonin and norepinephrine levels in the brain stem rose slowly, reaching about twice the normal value in 2 or 3 days (Fig. 2). By the third or fourth day a variable degree of central excitation was evident. Experiments in which the daily dose of iproniazid was 50 mg/kg elicited marked excitement in the animals on about the third day, when serotonin and norepinephrine levels had again risen about twofold. With a smaller daily dose of iproniazid (10 mg/kg), excitation was observed after 4 or 5 days, at which time

the brain levels of both amines were again about twice the normal values.

Isoniazid (isonicotinylhydrazine), a congener of iproniazid, is a poor inhibitor of amine oxidase. In daily doses of 50 mg/kg it caused neither a rise in the brain concentration of the amines nor any obvious pharmacologic effect. On the other hand, α -methyl, β -phenylethylhydrazine (JB 516, Lakeside Laboratories) a potent monoamine oxidase inhibitor of a different chemical series, when given daily in doses of 1 mg/kg, induced in 3 to 5 days pharmacologic effects similar to those seen after administration of iproniazid and raised the brain level of the amines about twofold.

It is not possible to conclude from the data given in this report that the central stimulatory effects of iproniazid are causally related to the increase in brain amines. It is noteworthy, however, that the administration of large doses of 3,4-dihydroxyphenylalanine (9), a norepinephrine precursor, or of 5-hydroxytryptophan (7), a serotonin precursor, causes central excitation which is enhanced by pretreatment with iproniazid.

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References and Notes

1. H. P. Loomer, J. C. Saunders, N. S. Kline, *Psychiat. Research Repts.*, in press.
2. E. A. Zeller *et al.*, *Experientia* 8, 349 (1952).
3. B. B. Brodie and P. A. Shore, *Ann. N.Y. Acad. Sci.* 66, 631 (1957).
4. P. A. Shore *et al.*, *Science* 126, 1063 (1957).
5. D. F. Bogdansk *et al.*, *J. Pharmacol. Exptl. Therap.* 117, 82 (1956).
6. P. A. Shore and J. S. Olin, *J. Pharmacol. Exptl. Therap.*, in press.
7. S. Undenfriend, H. Weissbach, D. Bogdansk, *Ann. N.Y. Acad. Sci.* 66, 602 (1957).
8. A. Pletscher, *Helv. Physiol. et Pharmacol. Acta* 14, C76 (1956).
9. A. Carlsson, M. Lindqvist, T. Magnusson, *Nature* 180, 1200 (1957).

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Inhibition of Adrenocortical Steroid Secretion by Δ^4 -Cholestenone

In the course of studies on Δ^4 -cholestenone as an inhibitor of cholesterol biosynthesis it was noted that rats fed high doses of the compound developed striking (six- to eight-fold) hypertrophy of the adrenal glands (1). The effectiveness of Δ^4 -cholestenone in depressing cholesterol synthesis (1, 2) and the demonstrated role of cholesterol as an intermediate in adrenal steroid synthesis (3) suggested that the latter might also be depressed. By direct measurement of cor-