administered intraperitoneally, so that initial high sulfadiazine levels could be obtained. Following the intraperitoneal injection, the mice were placed on the sulfadiazine diet for 24 hr. Similar results were obtained whether sulfonamide was administered only on the 6th or only on the 7th day following appearance of the vaginal plug. No data have been obtained concerning the period from the 1st to 6th day.

References

1. Copenhaver, W. M. and Detwiler, S. R. *J. exp. Zool.*, 1948, **109**, 2 9.
2. Detwiler, S. R., Copenhaver, W. M., and Robinson, C. O. *J. exp. Zool.*, 1947, **105**, 109.
3. Figge, F. H. J., Wolfe, G. F., and Figge, R. Y. *Anat. Rec.*, 1949, **103**, 121.
4. P'an, S. Y. *Proc. Soc. exp. Biol. Med.*, 1948, **68**, 488.

Enzymatic Reduction of 2,4-Diketo Acids Catalyzed by Dihydrodiphosphopyridine Nucleotide

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The reduction of pyruvic acid to lactic acid, catalyzed by dihydrodiphosphopyridine nucleotide ($DPNH_2$) in muscle extracts is well known. An apparently analogous reaction in which 2,4-diketo acids are reduced has been observed. When a series of homologous 2,4-diketo acids was incubated with 90% pure $DPNH_2$[1] and an aqueous extract of an acetone powder of rabbit muscle at pH 7.2, the characteristic absorption band of these compounds at 2900 A and the band due to $DPNH_2$ at 3400 A disappeared progressively. Equimolar amounts of coenzyme and diketo acid were utilized, suggesting the reduction of one keto group. In a typical experiment using a system containing 5×10^{-7} moles each of 2,4-diketovalerate and $DPNH_2$, 10^{-4} moles of phosphate buffer at pH 7.2, and 80 γ of protein nitrogen per 3 ml, 2.48×10^{-7} and 2.44×10^{-7} moles of $DPNH_2$ and diketo acid, respectively, disappeared after 12 min of incubation at 25° C. Neither $DPNH_2$ nor substrate disappeared when one of these was omitted from the system or in the absence of enzyme. No lactate was formed as determined by the method of Barker and Summerson (*1*), ruling out prior hydrolysis of the diketo acid to pyruvic acid (*3*). The facts are compatible with the equation,

$$RCCH_2CCOOH + DPNH_2 \rightarrow RCCH_2CHCOOH + DPN$$
$$\quad\;\; \| \quad \| \qquad\qquad\qquad\qquad\quad \| \quad |$$
$$\quad\;\; O \quad O \qquad\qquad\qquad\qquad\quad O \quad OH$$

whereby the product is considered tentatively to be the 2-hydroxy-4-keto acid.

The reaction proceeded more rapidly with increasing concentrations of substrate and was conveniently followed spectrophotometrically by measuring the rate of decrease of the $DPNH_2$ band at 3400 A. All of the normal 2,4-diketo acids from valeric to undecylic were reduced in the system, as shown in Table 1. Under the same experimental conditions, neither 4-keto valeric acid nor 3,5-diketohexanoic acid was reduced. The nature of the enzyme involved and its possible relationship to lactic dehydrogenase is under investigation.

TABLE 1

Enzymatic Reduction of 2,4-Diketo Acids*

2,4-Diketo acid	Disappearance of $DPNH_2$
	(Moles $\times 10^{-7}$ per min)
n-Valeric	1.83
n-Hexanoic	1.74
n-Heptanoic	1.45
n-Octanoic	1.48
n-Nonanoic	1.36
n-Capric	1.33
n-Undecylic	1.45

* Composition of system in moles per 3 ml was 5×10^{-7} $DPNH_2$, 9×10^{-6} diketo acid, 1×10^{-4} phosphate buffer (pH 7.2); and 0.1 ml enzyme preparation (100 γ protein nitrogen) per 3 ml; 25° C.

References

1. Barker, S. B. and Summerson, W. H. *J. biol. Chem.*, 1941, **138**, 535.
2. Hogeboom, G. H. and Barry, G. T. *J. biol. Chem.*, 1948, **176**, 935.
3. Meister, A. and Greenstein, J. P. *J. biol. Chem.*, 1948, **175**, 573.

A Convenient Quick Method of Obtaining Vitamin B_{12} Concentrate[1]

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The nonprotein filtrate of liver homogenate (proteins coagulated by boiling at pH 5.0) chromatographed on starch columns by the method of Moore and Stein (*1*) gave a reddish brown fraction in the first portions of the effluent.

The behavior and color of this fraction suggested a possible relation to B_{12}. This was tested as follows: 0.5 ml of liver injection, USP (Lederle Solution Extract, from beef liver, 15 u per ml) was dried by blowing air across it at room temperature. To the residue 0.1 ml 1*N* HCl was added, and then 0.5 ml of a mixture consisting of 0.1 *N* HCl, *n*-propanol, and *n*-butanol in the proportions 1: 2: 1. The solution was chromatographed on 25 g starch in a 10-mm × 300-mm column with the 1: 2: 1 mixture as solvent.

[1] This work is part of that done under a joint contract with the Office of Naval Research, United States Navy Department, and the United States Atomic Energy Commission.

[1] DPN was purified by countercurrent distribution as described by Hogeboom and Barry (*2*). Cruder preparations tended to interfere with measurements made at 2900 A.
m = calibration factor for adrenalin

The first 4.2 ml of the effluent was colorless. The next 1.8 ml, fraction 1, was slightly colored; the next 1.8 ml, fraction 2, was deep reddish brown; a third 1.8 ml, fraction 3, was slightly colored. No more of the effluent, up to a total volume of 22.2 ml, was colored.

The three colored fractions were submited to Karl Folkers, of Merck and Company, for analysis. He reported the following: fraction 1, 80 microbiological units per mg; fraction 2, 2000 units per mg; fraction 3, 240 units per mg.

As the color accompanies the activity, it is easy to know what portion of the effluent to collect. The method offers a way of obtaining B_{12} active material from commercially available sources, which already contain the activity in a conveniently small volume.

Reference

1. MOORE, S. and STEIN, W. H. *J. biol. Chem.*, 1949, **178**, 53.

Fat Absorption and Atherosclerosis[1]

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In our work on the effect of age on fat absorption (*1*) we have observed a phenomenon which may be fundamental in the pathogenesis of atherosclerosis.

Using a modification of Frazer's chylomicron dark field technique (*1, 2*), we have studied the absorption of fat in thirty young and in thirty old subjects with average ages of 18 and 76 years respectively. The fasting subjects were given a standard fat meal of ½ g of oleomargarine/kg of body weight on 2 oz of white toast together with a cup of tea. Samples of finger blood were drawn before and after the meal at regular intervals as indicated in Fig. 1. The number of chylomicrons in the serum of each specimen was determined and chylomicrographs were constructed.

As shown in Fig. 1, the chylomicron counts of young subjects reached a peak at 2½–3 hr, and returned to fasting levels by the end of the 5th hr. The counts of the old group on the other hand did not reach their peak until 8–12 hr, and they did not return to fasting levels until 24 hr had elapsed. In addition, the total number of chylomicrons was found to be consistently and considerably higher in the old than in the young group.

Previous work by Gage and Fish (*4*), and Frazer (*2*) has established that the chylomicron curve serves as an index of postabsorptive lipemia. From our results it is obvious that a definite delay in the rate of absorption and a definite increase in the total absorption of corpuscular fat exist in aged individuals as compared with a group of young subjects.

The observations of Hueper (*6, 7*) and of Moreton (*9, 10*) seem to indicate that the alimentary hyperlipemia and its accompanying high concentrations of chylomicrons

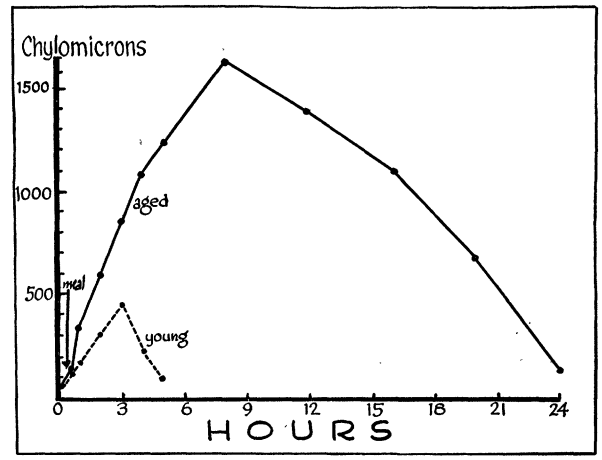

FIG. 1.

in the peripheral blood, occurring in normal individuals, are indistinguishable from the sustained hyperlipemia and hyperchylomicronemia of pathological and experimental origin which have been found to be characteristic of the known causative conditions of atherosclerosis.

Hueper (*6, 7*) and Moreton (*9, 10*) state that macromolecular substances can be deposited and can damage the internal layers of arteries. Chylomicrons are macromolecular bodies containing largely neutral fat and some cholesterol (*2, 3, 9, 10*). The neutral fat, according to Moreton (*9, 10*), disappears rapidly from the intima and subintima, while the cholesterol remains and accumulates gradually, attracting macrophages, giving rise to foam cells, and ending in atherosclerosis.

The question is still controversial, whether endogenous or alimentary cholesterol produces atherosclerosis in man. This cholesterol is in true solution and it is possible that chylomicrons, which are macromolecular aggregates, are the source of irritation and degeneration of arterial walls, rather than cholesterol, fats, or other lipids in the dissolved state (*6, 7, 9, 10*). In the chicken, endogenous cholesterol seems to play a large role in the genesis of arteriosclerosis (*5*).

Increased chylomicronemia following fat-containing food occurs at every age. However, it lasts only a relatively short time and it is only of moderate intensity in young persons. With increasing age, and particularly above 50 years of age, chylomicronemia is of greater intensity and it is practically permanent. If the chylomicrons play a role in atherosclerosis, the fundamental physiological basis of the mechanism of the disease may lie in this fact.

Another significant observation made was that oral ad-

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