The evidence obtained from the Japanese waltzing mice shows that within a closely inbred race, homiotransplants of splenic tissue may be quite as successful as autotransplants.

The experiments further show that *in all probability susceptibility to transplants of splenic tissue depends upon the same general principles of heredity found to apply in the case of tumor tissue—namely, multiple mendelizing factors.*

77 (1824)

Observations on cod-liver oil and rickets.

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In view of the nearly specific action that cod-liver oil has on rickets, it is of interest to inquire into the nature of the substance conferring on it the therapeutic properties. Several attempts have been made to isolate from it materials that could be made responsible for its action. Gautier and Morgues[1] isolated the organic bases contained in the oil and separated from them two alkaloid-like substances besides the simpler aliphatic amines. Funk[2] also worked with this mixture of bases which he fractionated in various ways. None of these observers, however, have published any data on the action of the isolated material. Stöltzner[3] claims, without giving any details of his evidence, except the statement that he cured even the worst cases of rickets, that hydroxy acids confer upon cod-liver oil its pharmacological properties. Freudenberg and Klocmann[4] had expressed similar ideas and prepared calcium salts of the unsaturated acids of cod-liver oil which they used in the treatment of spasmophilia.

Wacker and Beck[5] believe that "besides other chemically not yet well characterized substances, cholesterol plays a significant rôle in the antirachitic fat soluble factor A."

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[1] Gautier and Morgues, *C. R. Acad. Sci.*, 1888, cvii, 110 and 626.

[2] Funk, *Biochem. Bull.*, 1915, iv, 365.

[3] Stöltzner, *Münch. med. Wochenschr.*, 1921, lxviii, 272.

[4] Freudenberg and Klocmann, Jahresb. f. Kinderh., 1913, lxxviii, 47; 1914, xxix, 700.

[5] Wacker and Beck, *Berl. klin. Wochenschr.*, 1921, lxxxv, 453.

With a good test object now available in the rat made rachitic on the phosphorus low diet described by Sherman and Pappenheimer, the problem of determining the point in question is much easier. The rickets of children and the experimental rickets in rats both respond in the same manner to treatment with cod-liver oil[1] and there is no reason to believe that the substance active in the two cases should not be the same.

We first isolated the crude bases according to Gautier and Morgues and found them inactive. Next the oil was hydrolyzed with sodium hydroxide and the fatty acids separated. The fatty acids, when reasonably purified were entirely inactive, although in one of the first experiments a rather impure fatty acid fraction did slightly promote calcification. The residue of unsaponifiable matter gave a marked curative action. From this the bases were again isolated and these bases obtained after hydrolysis were also inactive. From the unsaponifiable matter in solution in alcohol a goodly portion of the cholesterol was crystallized out. This cholesterol fraction also was inactive. The material freed from most of the cholesterol was now more active than before. In this manner, we obtained fractions which on being diluted with ninety parts of cotton-seed oil, which had been found to be inactive, gave a curative effect a little stronger than the original cod-liver oil. The results were controlled by both X-ray of tibia and histological examination of rib sections.

This material also contains the fat soluble factor A, as we have been able to cure with it ophthalmia produced by deficiency of fat soluble. Very recently, Steenbock, Nelson and Hart[2] have made similar other extracts of saponified cod-liver oil and reported curative effects on ophthalmia in dogs. Although there is no longer any good reason to believe that rickets is a simple fat soluble deficiency,[3] the relation of the fat soluble A factor to the curative property of cod-liver oil remains to be worked out.

To sum up, we may say that the antirachitic substance of cod-liver oil can be demonstrated in the ether soluble "unsaponifiable"

[1] Shipley, Park, McCollum, Simmonds and Parsons, *Jour. Biol. Chem.*, 1920–21, xlv, 343.

[2] Steenbock, Nelson and Hart, *Am. Jour. Physiol.*, 1921, lvii, 14.

[3] Pappenheimer, McCann and Hess, *Jour. Biol. Chem.*, 1921, xlvii, 395.

[4] Shipley, McCollum and Simmonds, *Jour. Biol. Chem.*, 1921, xlix, 399.

fraction after alkaline hydrolysis. It is not an organic base of the type described as occurring in cod-liver oil. It is not cholesterol, but similar to cholesterol in its solubilities. The suggestion is made that it may be a sterol related to cholesterol or a cholesterol derivative. The fatty acids of cod-liver oil are entirely inactive in curing rickets.

78 (1825)

The distribution of inorganic phosphate of the blood between plasma and cells.

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Since Greenwald's work on the organic acid soluble phosphorus our knowledge of the various phosphorus compounds in the blood is steadily increasing and acquiring significance. The determination of the inorganic blood phosphate, however, has been very questionable, particularly in corpuscles, due to the ease with which the organic acid soluble phosphate is hydrolyzed. Any method which requires considerable time or in which the phosphate has to be precipitated, or in which the red cells are washed or otherwise manipulated, comes very gravely under suspicion of having allowed a significant amount of hydrolysis to take place.

When working only with plasma these precautions are not so necessary. Bloor's[1] figures for inorganic phosphate in the corpuscles are admittedly high. A method very well suited to estimation of inorganic phosphate is that of Bell and Doisy[2] in which the color of the blue reduction product of phosphomolybdic acid is measured as in Folin's uric acid and phenol determintion, the limiting factor, however, being the phosphate.

Our results have been briefly as follows:

When working rapidly with the Bell and Doisy method, the inorganic phosphate in the plasma and the whole blood is the same within the limit of error of the method. The few exceptions to this were traced to improper handling of the blood or too long a time elapsing before the determination. Even here the whole

[1] Bloor, *Jour. Biol. Chem.*, 1918, xxxvi, 49.

[2] Bell and Doisy, *Jour. Biol. Chem.*, 1920, xliv, 55.