TABLE 2

THE EFFECT OF AN INCREASE OF PANTOTHENIC ACID IN THE EGG ON THE VITAMIN LEVEL OF THE LIVER, BRAIN AND HEART OF THE DAY-OLD CHICK

Vitamin	Vitamin level of eggs raised by supplementing diet of hens								
	Exper. liver γ/gm.*	Control liver γ/gm.	Exper. liver when control = 100	Exper. brain γ/gm.	Control brain γ/gm.	Exper. brain when control = 100	Exper. heart γ/gm.	Control heart γ/gm.	Exper. heart when control = 100
Pantothenic acid ...	98.0	99.0	99.0	270.0	250.0	108.0	224.0	186.0	120
Inositol	2600.0	2200.0	118.0	8800.0	13000.0	67.5	2700.0	2400.0	113
Nicotinic acid	260.0	410.0	63.5	200.0	230.0	87.0	262.0	246.0	107
Riboflavin	40.0	51.0	78.5	6.3	6.9	91.2	32.0	34.0	94
"Folic acid"	99.0	180.0	55.0	5.0	7.5	66.7	6.6	6.0	110
Thiamin	5.2	5.7	91.0	2.5	5.3	47.0	2.8	2.9	97
Pyridoxin	1.4	2.4	59.2	2.5	1.3	192.0	.98	.77	127
Biotin	.50	.39	128.0	.028	.038	74.0	.078	.075	104

* γ/gram dry weight.

mental embryos were examined for blood hemoglobin concentration and relative size of the heart and brain. Data on the effect of the varied pantothenic acid level in the egg on hatchability were also obtained.

Six control and six experimental eggs from the group with the highest level of pantothenic acid were allowed to hatch and the liver, heart and brain of these chicks were assayed for several of the "B vitamins."

It is evident from Table 1 that there was a definite tendency for increased pantothenic acid in the egg to be associated with an increased hemoglobin concentration of the blood in the 12-day chick embryo. The effect on the relative brain and heart size depended upon the concentration of pantothenic acid present in the egg. A comparatively low concentration tended to be associated with a larger than normal brain, while both heart and brain were depressed in relative size in the embryos from the eggs with higher levels of pantothenic acid.

Embryo survival was better in every group of the eggs from the hens on the supplemented diet, indicating that a relatively high level of pantothenic acid in the egg is associated with improved hatchability.

The results given in Table 2 show that concomitant with the changes in brain and heart size there was also some shift in the balance and level of the eight vitamins in the tissues for which assays were made. This was especially true for the liver and the brain.

Work in progress indicates that some other members of the "B complex" modify the chick development when their level in the egg is raised to a moderate degree—which tends to confirm the results reported here for pantothenic acid.

It appears that during early embryological development, the chick embryo is highly responsive to a vitamin imbalance created by a moderate increase in the level of one of these food elements. Apparently the embryo is dependent on this vitamin balance even for such fundamental characteristics as blood hemoglobin concentration and brain and heart size.

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THE PHENOMENON OF THIXOTROPY IN HEMOPHILIC AND HEPARINIZED BLOOD[1]

IN 1923 Szegvari and Schalek[2] observed a property of gels with hydrous ferric oxide for which the term "thixotropy" was suggested by Péterfi in 1927.[3] Ever since it has often been observed that an apparently solidified gel or a very viscous colloid can be liquefied by shaking or other mechanical agitation and upon standing again becomes a gel. This process, in which the time factor becomes apparent, can be repeated and is dependent upon temperature. Previous to this observation in Freundlich's laboratory[2, 4] Chambers[5] had observed in 1921 that frequently protoplasm becomes fluid by stirring with the micro-manipulator needle, and after some time becomes gelatinous again when permitted to stand. It is of great interest and it should be recognized that the phenomenon of thixotropy was described for the first time in 1910 by Howell[6] in studies of the conversion of fibrinogen to fibrin by the addition of thrombin in various amounts. He referred to observations on a similar process in horse's plasma at an earlier time.[7] Minot and Lee[8] observed this

[1] Aided by a grant from the Dazian Foundation for Medical Research.

[2] A. Szegvari and E. Schalek, *Kolloid-Z.*, 32: 318, 1923; 33: 326, 1923.

[3] T. Péterfi, *Arch. f. Entwcklngsmech. d. Organ.*, 112: 660, 1927.

[4] H. Freundlich, *Kolloid-Z.*, 46: 289, 1928.

[5] R. Chambers, *Proc. Soc. Exp. Biol. and Med.*, 19: 87, 1921.

[6] W. H. Howell, *Am. Jour. Physiol.*, 26: 453, 1910.

phenomenon with blood coagula of hemophiliacs and called it "reclotting phenomenon." There is only occasional reference made to it in the literature on hemophilia and it has not been especially studied by any one.[9]

Minot and Lee, who never wrote further on this phenomenon, at least in the direct sense,[10] described thixotropy as follows: "If, after the hemophilic blood had apparently clotted firmly, the clot was loosened and removed from the fluid, the fluid would clot and would do so sometimes almost at once, often in three to five minutes and sometimes not for ten to thirty minutes or more. Again on separating the clot, but not removing it from the serum, this reclotting phenomenon occurred and might be repeated from three to six times. The reclotting would occur no matter whether the clot was loosened a few minutes or a few hours after it had appeared solid. If one waited hours rather than minutes after the clot had formed, one usually obtained the reclotting phenomenon fewer times." Since we[11] succeeded in producing experimentally a hemophilia-like condition in mice by injections of excessive doses of purified heparin, it was of interest to investigate if thixotropy would occur with coagula of blood heparinized *in vitro* and *in vivo*.

Blood was obtained from the antecubital vein of humans and the femoral and jugular veins and the femoral artery of dogs. Heparin of the Connaught Laboratories, University of Toronto, 110 units per milligram, was used in doses between 0.1 and 2.0 units in physiological saline. All experiments were done at room temperature. 0.02 cc of the heparin solutions were placed into clean test-tubes and 1 cc of blood was added to each tube. In intervals between 30 seconds and 1 hour after the clot had formed it was disturbed by shaking or stirring and the time was measured until the clot formed again. For controls, blood was added to 0.02 cc of physiological saline. Blood coagula were tested from 44 dogs and 6 humans. Thixotropy always occurred with blood which was mixed with heparin, whereas the phenomenon never occurred in the controls. In samples containing 0.4 to 0.5 units, thixotropy occurred two to four times within 1 hour following the coagulation time of the blood. The phenomenon, if reproducible, occurred after a longer interval of time than previous to it after the second successive reclotting. Blood from 12 dogs drawn 26 to 50 minutes following intravenous injection of 200 units of heparin per kilogram weight was tested for its thixotropic property. In all cases thixotropy occurred two to seven times.

The observations of Minot and Lee could be repeatedly and fully verified with blood coagula of two hemophiliacs. The phenomenon was likewise observed with hemophilic plasma which was prepared after centrifuging hemophilic blood at 3,000 r.p.m. for 15 minutes. After the plasma had coagulated, the gel was loosened by a glass rod upon which it retracted rather suddenly and the formed fibrin was removed. From one or several minutes the gel formed again and the process of thixotropy could be repeated; in one case for five, and in the other for eight times. Plasma which was obtained from three of the heparinized dogs showed similar results, although it occurred only for two to three times.

The occurrence and frequency of thixotropy with heparinized blood or plasma is dependent upon the amount of heparin, the coagulation time and the length of time following the coagulation time and the reclotting, after which the gel is liquefied by mechanical agitation. The results indicate that the fibrinogen is progressively but slowly, at times over a period of hours, converted into fibrin in both the heparinized and the hemophilic conditions. This process may occur as long as there is either thrombin forming or until the amount of fibrinogen is exhausted due to its conversion. Studies of the mechanism involved in the production of thixotropy with blood coagula are being done. We are also determining whether the same mechanism is responsible for the phenomenon with both the hemophilic and heparinized coagula.

"Thixotropy" may be considered as a general term describing a physical system going for several times from semisolid to liquid state on mechanical agitation. It must be taken for certain that protoplasm with definite fibrillar elements actually exists according to Jordan.[12] That these protoplasmatic structures may be similar to the fibrin needle network of plasma gels is suggested at this time; however, the degree of similarity can only be taken into account by future work. Since a more exact classification of thixotropic phenomena does not exist at present the use of this term seems to be justified. The inclusion of the "reclotting phenomenon" with miscellaneous thixotropic systems should stimulate interest and ultimately lead to a better understanding of the thixotropy in blood plasma.

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[7] Professor Howell states in a personal communication that this work was done as early as 1892.

[8] G. R. Minot and R. I. Lee, *Archiv. Int. Med.*, 18: 474, 1916.

[9] W. H. Howell, personal communication, 1941.

[10] G. R. Minot, personal communication, 1941.

[11] A. L. Copley and J. J. Lalich, *Am. Jour. Physiol.*, in press.

[12] H. J. Jordan in "First Report on Viscosity and Plasticity," Amsterdam and New York, Nordemann Publishing Company, 1939.