casein or of gelatin in these experiments would be purely speculative at this point. It seems impossible that a deficiency of an essential amino acid could be involved in odoratism because the animals continue to grow. Further, the symptoms of odoratism are profoundly different from those produced by any known amino acid deficiency.

*Summary.* High levels of gelatin and of casein delayed, but did not prevent, the onset of odoratism (experimental lathyrism) in the rat. Casein exerted a greater protective action than gelatin.

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## Macrocytic Anemia and Leucocytosis of Guinea Pigs with Muscular Stiffness Disease.* (20928)

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A deficiency syndrome characterized by muscular stiffness was described by Wulzen and Bahrs(1) in guinea pigs raised on skim milk diets supplemented by minerals and the known vitamins. Physiological, chemical, and pathological aspects of the disease were studied(2-6).

The present investigation was undertaken to determine whether hematological changes occurred in guinea pigs deficient in the anti-stiffness factor. Preliminary results of these studies have been published(7).

*Methods.* Guinea pigs used to establish the normal blood values were segregated as to sex and raised on diet of rolled barley, liberal amounts of kale or grass, iodized salt, and wheat straw *ad lib.* None showed wrist stiffness. Gravid animals were not tested. Experimental animals were fed the following diet deficient in the anti-stiffness factor(8). A.M.: skim milk powder 20.0 g, water 80.0 g, copper sulfate 0.078 mg, ferric chloride 0.048 mg, ascorbic acid 10.0 mg; P.M.: skim milk powder 20.0 g, water 80.0 g. Fat- and water-soluble vitamins were added alternately to the evening diet to make the average daily consumption per animal for each vitamin as follows: thiamin hydrochloride 0.20 mg, pyridoxine hydrochloride 0.10 mg, riboflavin 0.50 mg, nicotinic acid 1.0 mg, pantothenic acid 0.10 mg, inositol 10.0 mg, para-aminobenzoic acid 2.0 mg, choline 50.0 mg, biotin (SMA conc. S200) 0.01 mg, beta-carotene 150 I.U., viosterol 40 I.U., alpha-tocopherol 0.10 mg, 2-methyl-1,4-naphthoquinone 0.10 mg, folic acid 0.003 mg. All received straw and iodized salt *ad lib.* Blood samples were taken from the ear which was gently stroked or warmed to cause an hyperemia(9). Incisions were made with Bard-Parker "11" blades. The animals were tame from frequent handling in the laboratory and did not have to be confined in any way when samples were taken. Erythrocyte counts were made after diluting the blood with Toisson's solution. Hemoglobin estimations were made with the Sahli-Haskins technic (10). The hemoglobin coefficient, according

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to Osgood(10), was calculated as the amount of hemoglobin per 100 cc of blood if the count is adjusted to 5,000,000 per cu mm. Average red cell diameters were measured with the Haden-Hauser Erythrocytometer(11,12). No variation of more than one-fifth micron from slide to slide for any one animal was found in these experiments. The elimination of any slide not having a bright, clear halo is probably the reason for this uniformity. Basophilic aggregations were used to determine the percentage of young erythrocytes in the circulating blood. The method is based on the finding by Pearlman and Limarzi of a close correlation between basophilic aggregation and reticulocyte levels in several diseases and a similar response of basophilic aggregations and reticulocytes to liver therapy in pernicious anemia(13). Sedimentation rates were determined with the Landau-Adams microsedimentation apparatus(14). White cell counts were obtained from blood collected in a Thoma white cell pipette and diluted 1:10 with 3% acetic acid. Differential count smears were made with a narrow slip (about 3/8" wide) of parchment replacing the customary second glass slide. This procedure eliminated to a large extent the clumping and distortion of guinea pig white cells along the edges of the smear which occurred when a glass slide was used for smearing. Cells were counted at a point where the red cells did not touch, and the slide was counted from edge to edge until 200 or more cells had been differentiated.

TABLE I. Effects of Deficiency of Anti-Stiffness Factor on Erythrocytes of Guinea Pigs.

| Diet* | No. weeks on diet | No. animals | Mean blood value ± stand. dev. |
|---|---|---|---|
| | | | (Red cell count) |
| S | 20–130 | 40 | $4.9 \times 10^6 \pm 1 \times 10^5$ |
| D | 24–120 | 53 | $4.2 \times 10^6 \pm 8 \times 10^5$† |
| DM | 90‡ | 5 | $5.0 \times 10^6 \pm 3 \times 10^5$ |
| | | | (Hemoglobin) |
| S | 20–130 | 16 | 15.4 ± .9 g |
| D | 26–110 | 53 | 10.9 ± 2.0 † |
| DM | 90‡ | 5 | 13.4 ± .9 † |
| | | | (Hemoglobin coeff.) |
| S | 20–130 | 16 | 15.6 ± 1.0 g |
| D | 26–110 | 53 | 13.3 ± 1.8 † |
| DM | 90‡ | 5 | 13.3 ± 1.0 † |
| | | | (Red cell diam.) |
| S | 20–130 | 39 | 7.35 ± .13 μ |
| D | 30–110 | 46 | 7.69 ± .22 † |
| DM | 90‡ | 5 | 7.32 ± .13 |
| | | | (Basophilic aggregations) |
| S | 20–130 | 25 | 2.06 ± .41% |
| D | 3– 10 | 19 | .98 ± .52 † |
| D | 21– 74 | 51 | .68 ± .28 † |
| DM | 90‡ | 5 | 2.20 ± .79 |
| | | | (Micro sedimentation) |
| S | 20–130 | 31 | 2.35 ± .68 mm |
| D | 10– 45 | 58 | 3.96 ± 2.87 † |
| D | 50– 84 | 27 | 3.02 ± 1.96 |
| DM | 90‡ | 5 | 1.92 ± .69 |

* S = Stock; D = Deficient; DM = Deficient + molasses.

† Statistically significant at .01 level. All groups compared with stock animals except where otherwise indicated.

‡ Fed deficient diet for 9 mo; then deficient diet plus molasses for 1 yr.

*Results.* I. *Erythrocytes.* A summary of the data collected on red blood cells is presented in Table I.

In general, deficiency of the anti-stiffness factor produced a mild to severe anemia. Twenty-five per cent of the deficient animals had red cell counts of $3.5 \times 10^6$ to $2.2 \times 10^6$ and hemoglobin of 9.6 to 5.9 g. The hemoglobin per cell decreased for the deficient animals. Fifty-two per cent of the animals had red cell diameters ranging from 7.7 to 8.2 μ.

Accompanying the anemia was a decrease in the number of young red cells of the circulating blood. This effect appeared as early as 3 to 10 weeks. High sedimentation rates occurred as early as 2 to 6 weeks of deficiency. The microsedimentation rates for guinea pigs on the deficient diet for 10 to 45 weeks varied from 2.0 to 19.0 mm per hour. However, animals that had been deficient for 50 to 84 weeks had an average sedimentation rate that was not significantly different from the normal. However, the range for the microsedimentation rates of these older deficient animals was 0.5 to 8.7 mm per hour.

In general, these older deficient animals fall into 2 classes of symptoms of deficiency of the anti-stiffness factor: 1. A small group of older healthy, robust animals that seem to be highly resistant to deficiency of the anti-stiffness factor. They usually have stiff wrists without soreness. Without exception these animals sooner or later die from deficiency of the anti-stiffness factor. 2. A large group of

TABLE II. Differential Count. Guinea pigs deficient in the anti-stiffness factor for periods of 24 weeks compared with stock guinea pigs.

| | Cell type | 10 stock ♀ | 10 stiff ♀ | 11 moderately stiff ♀ | Avg all stiff ♀ | 24 stock ♂ | 55 stiff ♂ | 9 moderately stiff ♂ | Avg all stiff ♂ |
|---|---|---|---|---|---|---|---|---|---|
| | | Deficient females compared with stock females (mean %) | | | | Deficient males compared with stock males (mean %) | | | |
| I. | Granulocytes | 21.89 | 38.88† | 24.65 | 31.43§ | 25.41 | 50.21* | 55.36* | 50.94* |
| | Neutrophils | 19.00 | 22.42 | 22.71 | 22.57 | 23.87 | 43.23* | 54.74* | 44.88* |
| | Eosinophils | 2.53 | 16.53* | 1.54 | 8.28 | 1.13 | 6.09* | .30 | 5.25* |
| | Basophils | .32 | .88 | .41 | .63 | .40 | .79 | .32 | .72 |
| II. | Agranulocytes | 72.95 | 58.41† | 72.29 | 65.58 | 69.17 | 46.40* | 41.59* | 45.71* |
| | Lymphocytes | 69.43 | 55.18‡ | 70.41 | 63.16 | 65.78 | 44.98* | 39.34* | 44.17* |
| | Monocytes | 1.25 | 1.10 | .69 | .88 | 1.53 | 1.32 | 1.68 | 1.37 |
| | Kurloff | 2.84 | 1.97 | .89* | 1.40† | 1.81 | .00* | .03* | .00* |
| III. | Disintegrated | 4.64 | 2.58 | 3.00 | 2.80§ | 5.33 | 2.91† | 2.95 | 2.91* |

* Significant at .001 level. † Significant at .01 level. ‡ Significant at .02 level.
§ Significant at .05 level.

older animals with varying degrees of abnormalities in growth, stiffness, body contour, calcification, tooth growth, and hearing(3). All of the high sedimentation rates among the older animals were found in this latter group of animals.

Five guinea pigs that had been subjected to a deficient diet plus a protective supplement of molasses for one year had normal values for red cell counts, red cell diameters, basophilic aggregations, and sedimentation rates. Hemoglobin was statistically significantly higher than the hemoglobin for the deficient animals, but had not returned to a normal level. The hemoglobin coefficient was lower than normal. New cells were being formed but the hemoglobin per cell was low.

II. *Leucocytes.* White cell counts were made on 52 deficient guinea pigs and on 57 stock guinea pigs. The deficient guinea pigs showed a leucocyte count of 16690 (standard deviation 6422) compared with the white cell count of 7680 (standard deviation 2049) for non-deficient guinea pigs. A few counts were as high as 30000 and 40000. Usually the leucocyte counts did not become elevated until about 3 to 5 months of deficiency. The 5 guinea pigs fed the deficient diet for 9 months and the deficient diet plus a molasses supplement for one year had normal leucocyte counts with an average value of 8340. The white cell differential count indicated an increased percentage of granulocytes and a decreased percentage of agranulocytes. However, the total number of both cell types actually increased. The data has been analyzed further by dividing the deficient animals into 4 classes: stiff females, moderately stiff females, stiff males, and moderately stiff males (Table II).

Stiff male and female guinea pigs had a persistent eosinophilia. In every case eosinophilia was associated with moderate or severe stiffness. In only 2 cases out of 85 was severe stiffness not accompanied by eosinophilia, and one of those cases was a 6% basophilia. Stiff males also had a markedly increased percentage of neutrophils in contrast to the stiff females.

In a few cases there were animals that did not become extremely stiff although they had received the diet for 24 to 110 weeks. However, calcification of musculature was frequently prominent. The females of this group showed little change from normal with only a decrease in Kurloff(15) cells. Males of this group had higher percentages of neutrophils than did the stiffer male guinea pigs. The total percentage of granulocytes was relatively the same for the 2 groups. An interesting finding was the complete disappearance of the Kurloff cell type in deficient male guinea pigs.

*Summary.* 1. Deficiency of the anti-stiffness factor in guinea pigs produced anemia characterized by decreased numbers of red cells, less hemoglobin per cell, and macrocytosis. The number of young red cells also decreased. The sedimentation rate was in-

creased. 2. The white cell count rose markedly with an increase in all cell types. The differential count indicated a granulocytosis. The Kurloff cell disappeared in deficient males.

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## Paroxystic Activity of Deplanted Nerve Centers in Amphibia, as Influenced by the Ionic Environment.* (20929)

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Numerous investigations in neurobiology have made use of the facilities provided by Urodele larvae whose nervous system can be submitted to a wide variety of experimental procedures. Among these, the method of "deplantation" recently developed by Weiss (1,2) deserves careful consideration because, among other advantages, it provides a completely isolated pool of neurons which can be studied under simple conditions. A fragment of spinal cord or medulla of Amblystoma or Triturus can thus be deplanted into the dorsal fin of another larva where it will grow and reorganize for long periods of time. If a limb is grafted nearby, it will receive motor innervation from the neural deplant and so become an effector displaying the activity going on in the deplant. A striking result of these experiments was that the isolated deplant does not remain quiescent; it develops endogenous ("spontaneous") paroxystic discharges which are seen as twitches or tonic-clonic fits exhibited by the grafted limb at irregular intervals. The phenomenon can no doubt be put in line with other evidence suggesting that autorhythmicity is a fundamental property of nervous elements (Fessard(3)). Such a statement, however, does not solve the problem, because this type of activity is not observed in freshly deplanted spinal segments(1) and one has still to consider how it is released after a few days' isolation. Evidence of structural redistribution and "randomization" of neurons within the deplants(2) favors the idea of a liberation from the "harnessing influence lying in the organization of the intact nervous system" (1). Put in other words, this endogenous activity might well be related to the phenomena of sensitization by denervation. In-

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