that the general conclusion must be drawn from the experiments that, riboflavin, nicotinamide, thiamin chloride, pyridoxine, calcium pantothenate, folic acid and biotin produced a negligible effect upon growth; that is, proliferation of cells was not enhanced.

A much more interesting observation noted was the effect of the B vitamins upon *survival* of the outgrowths. Many cultures, regardless of the rate of proliferation, outlived their controls for some time after all growth was static. In other words, they did not degenerate as rapidly even though active growth had ceased. This was most noticeable in brain cultures with biotin and folic acid in which the controls were badly degenerated after 5 days whereas the vitamin cultures, especially those containing folic acid, survived an additional 7 days.

It is common knowledge among tissue culturists that to maintain growth and multiplication of cells in culture, one must supply a medium containing some stimulating substance, the best of which is embryo tissue extract.[7] Cultures grown in media without embryo extract grow only to a limited extent and degenerate rapidly as compared with cultures of the same tissues grown with embryo juice. Since the B vitamins investigated here in certain concentrations produced equivalent growth in cultures of brain, skin, and heart where embryo juice was not employed it seems probable that they may, in part at least, be responsible for supplying some of the growth factors of tissue extracts.

It is interesting that data from the Texas group of investigators[8] on the analysis of beef heart and beef heart nuclei for all of the B vitamins indicate that the content of nuclei for each vitamin was 3 to 4 fold greater than for the cytoplasm except for pyridoxine which was slightly less and biotin which was about half as much. If such analyses hold true for other tissues in general it might be suggested that, with the exception of biotin and pyridoxine, the B vitamins might be essential for maintaining the integrity of the nuclei; biotin and pyridoxine, for cytoplasmic effects. Since the nucleus is considered paramount for the maintenance of the cell as a living unit, might the longevity noted in some of the vitamin cultures over their controls be due to an effect principally upon the nuclei?

Summary. Cultures of skin and brain (ectodermal derivatives) and heart (mesodermal derivative) in the presence of the B complex vitamins in certain concentrations, grew as well as, or slightly better than their own controls without vitamins. From the results of all the experiments the conclusion must be drawn that the added B complex vitamins have a negligible growth stimulating effect upon tissue cultures of skin, brain and heart. However, they did seem to affect favorably the longevity of the cultures, particularly those of brain, with biotin or folic acid. These outlived the majority of their controls in good (biotin) and excellent (folic acid) condition by a span of days more than once again as long.

[7] Ebeling, A. H., *J. Exp. Med.*, 1913, **17**, 273.

[8] Isbell, Edith R., Mitchel, Herschel K., Taylor, Alfred, and Williams, Roger, *Univ. of Texas Publ. No.* 4237, 81-83.

15463 P

Production of Atheromatosis in the Aorta of the Chicken by Administration of Diethylstilbestrol.*

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The experimental production of a hyperlipemic atheromatosis by methods other than the feeding of cholesterol has not hitherto been reported. It is shown here that atheromatosis can be induced in the bird by the subcutaneous implantation of diethyl-

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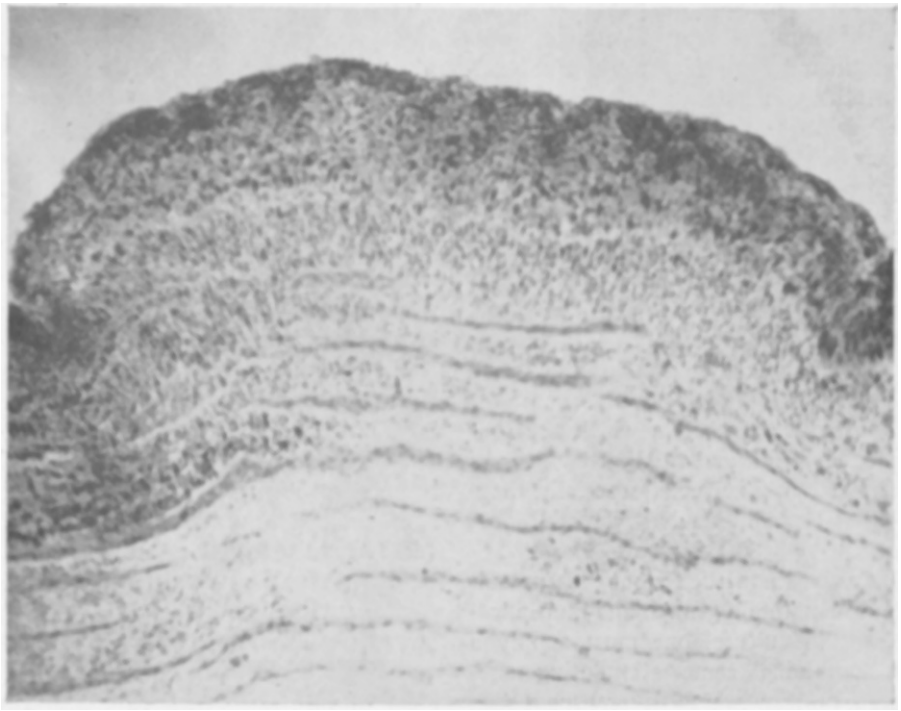

Fig. 1.
Thoracic aorta of Bird 1548. Sudan IV × 120.

stilbestrol, a procedure that results in a sustained hyperlipemia.[1] This provides a new and simple procedure for the study of experimental atheromatosis. It has the distinct advantage in that aortic lesions so produced more closely resemble the spontaneous lesions in birds than do those produced by the exogenous administration of cholesterol.

Pellets of diethylstilbestrol, each weighing approximately 25 mg, were implanted into White Leghorn cockerels obtained from a single hatch. A single pellet was injected into each bird when it was 3.3 months old and at the following intervals thereafter: 30 days, 40 days, and 65 days. Blood samples were taken for lipid analyses at monthly intervals during the course of the experiment. All birds used in this study were fed a stock diet, the composition of which has been described elsewhere.[2] The birds were sacrificed by exsanguination and their tissues examined histologically.

Thoracic Aorta. The thoracic aortas of 5 of the 7 birds that were examined from 6 to 7 months after implanation of the first pellet contained lesions that were grossly visible. These were lemon yellow in color and appeared as elliptical plaques that measured from 1 to 2 mm in width and from 12 to 15 mm in length. In 4 of the birds these areas were slightly elevated. They were most numerous on the convex side of the aortic arch, although in one bird they were also found in the brachiocephalic arteries and in the lower thoracic and abdominal portions of the aorta.

Microscopic lesions of the aortic arch were found in all 7 of the diethylstilbestrol-injected birds. These consisted of zones of intimal thickening elevating the endothelium; when extensive enough, this process produced the visible plaques (Fig. 1). The thickened intima was composed of large vesicular fibroblastic cells supported by reticulum and

1 Entenman, C., Lorenz, F. W., and Chaikoff, I. L., *J. Biol. Chem.*, 1940, **134**, 495.

2 Lorenz, F. W., Chaikoff, I. L., and Entenman, C., *J. Biol. Chem.*, 1938, **123**, 577.

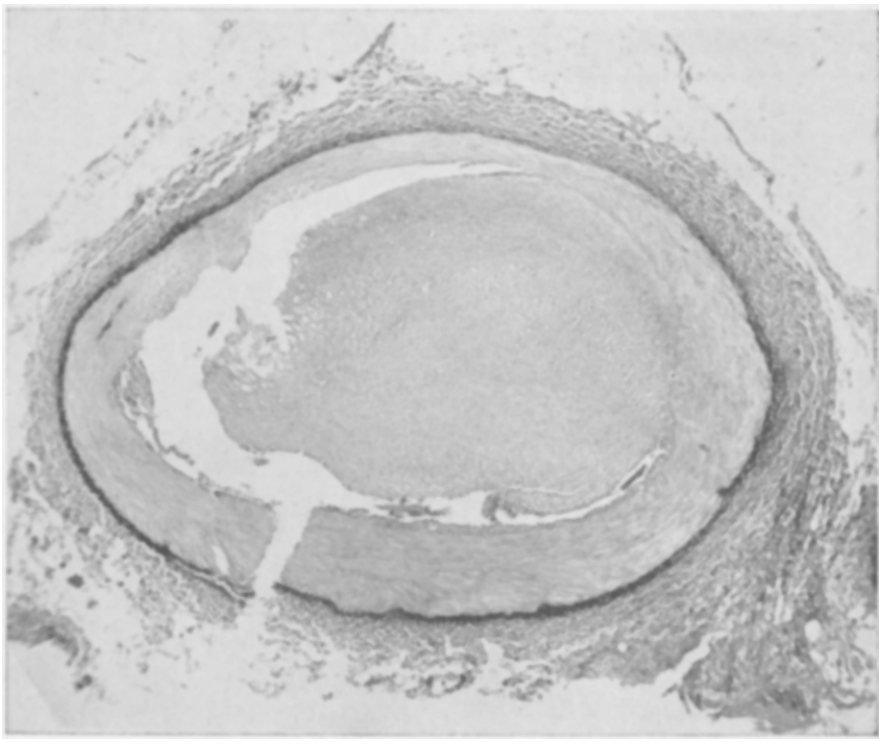

Fig. 2.
Abdominal aorta of Bird 1565. Weigert-Van Giesen × 36.

collagenous fibers that lay perpendicular to the endothelial surface. A few foam cells of the macrophage type were found in these intimal plaques. The intimal thickening was produced not only by an infiltration of lipids but also by an increase in the number of connective tissue cells. The cytoplasm of the connective tissue cells of the inner portion of the media and of those parts of the intima that were not thickened was also foamy and vesicular.

By the use of Sudan IV and Nile blue sulfate stains it was demonstrated that the cytoplasm of all connective tissue cells and macrophages in the intima and of all connective tissue cells of the adjacent media of the aortic arch was distended by small lipid droplets (Fig. 1). The bulk of this material was not cholesterol, although moderate numbers of refractile crystals (cholesterol) were visible with polarized light.

Nine control birds were sacrificed at 11 months of age. In 8 no gross or microscopic alterations were found in the thoracic aorta. In a single bird a negligible number of minute lipid droplets were observed in a few of the connective tissue cells of the intima and adjacent media in the arch of the aorta.

Abdominal Aorta. Eight of the 9 control birds that were sacrificed at the age of 11 months showed gross or microscopic evidence of the "spontaneous" lesion of the abdominal aorta that has been described for the domestic fowl.[3] In 4 of the birds there were longitudinal, elongated ridge-like plaques on the anterior wall just above the bifurcation. They measured 1-2 mm in width and 7-15 mm in length and were pearly grey in color. Microscopic examination showed that they consisted of dense hyalinized connective tissue fibers lying parallel to the circumference of the vessel and enclosing vesicular fibroblasts. These cells in the deeper and central portion of the plaques were filled with lipid droplets. Small amounts of lipid material were also

[3] Dauber, D. V., *Arch. Path.*, 1944, **38**, 46.

present in the adjacent media.

Although the "spontaneous" lesions were also found in the stilbestrol-injected birds, they differed from those found in the controls in that they contained an abundant amount of lipid material some of which was cholesterol. Moreover, degenerative changes as well as calcification had occurred in the plaques of the stilbestrol-treated birds (Fig. 2).

In none of the control birds did the level of total lipids of plasma exceed 500 mg per 100 cc. Although considerable fluctuations occurred in the lipid level of the plasma of the stilbestrol-treated birds, values for total lipids well over 10,000 mg per 100 cc were commonly found; in a single bird the concentration of total lipid rose to 17,800 mg. Neutral fat accounted for most of the rise in total lipid. In the control birds the levels of total cholesterol in plasma did not exceed 130 mg, whereas in the injected group values above 500 mg were frequently observed.

15464 P

A Transitory Decrease in Glucose Tolerance Following Experimental Lesions in the Central Nervous System.*

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In the process of determining daily postoperative blood sugars in animals with major traumatic experimental lesions in the brain stem, it was noted that an immediate postoperative hyperglycemia seldom occurred,[1,2] but in some instances a definite hyperglycemia appeared after the onset of postoperative feeding, *i.e.*, when blood sugar was determined 18 to 24 hours after the previous meal.

It seemed apparent that this postfeeding hyperglycemia was due to the presence of a decrease in the animal's tolerance for carbohydrates, the 18- to 24-hour hyperglycemia being the direct result of an excessive prolongation of the meal tolerance curve. For the purpose of ascertaining if this were true, the glucose tolerance in dogs has been followed for extended periods after major intracranial procedures.

Methods. The intracranial procedures consisted of (1) hemisection and transection of the midbrain and pons, (2) destruction of the ventral tuberal portion of the hypothalamus, and (3) removal of the cerebellar vermis and the cerebellum in its entirety. Operative procedures were carried out under sodium pentobarbital anesthesia (30 mg per kg of body weight), and have been described in detail elsewhere.[3-5]

The glucose meal consisted of 2 g per kg of body weight, administered in a 20% aqueous solution by stomach tube. Tests were run 24 hours after the previous meal, and postoperative feeding was begun the day after operation, *i.e.*, 48 hours after the last preoperative meal. Blood sugar was determined by the Gibson modification of the Folin-Wu method.[6]

Results. Elevated and prolonged tolerance curves, ranging from hardly detectable (see Dog 3-C, Fig. 1) to very striking effects (see Dogs 5 and 4-C, Fig. 1) were routinely encountered following these procedures. This

* Aided by a grant from the John and Mary R. Markle Foundation.

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3 Keller, A. D., Roy, R. S., and Chase, W. P., *Am. J. Physiol.*, 1937, **118**, 720.

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5 Keller, A. D., and Hamilton, J. W., Jr., *Arch. Surg.*, 1938, **37**, 760.

6 Gibson, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 480.