a priori to be the most likely. In order to substantiate this hypothesis it was necessary to provide a reasonable explanation for an initial stimulation of the cortical activity under reduced pressure. A greater demand for cortical hormone by the body under diminished pressure appeared as the most reasonable explanation. Attempts to obtain some evidence for an increase of the cortin requirements of low pressure rats constituted the third step in this investigation.

The cortin requirements of adrenalectomized rats were first carefully determined with respect to atmospheric conditions. This procedure was repeated with respect to a series of graded low pressure environments. The amounts of assayed charcoal adsorbates of cortical hormone needed to maintain the adrenalectomized low pressure rats alive increased progressively with the lowering of the pressure gradient. Moreover, the increment in rat units per 100 mm. decrease in pressure underwent a gradual and quite considerable rise with the lowering of the pressure. The minimum dose was more than 20 times higher at a pressure of 300 mm. than normally. It may be inferred that rats with intact adrenals under a similar pressure require an equal amount of cortical hormone and that their cortical apparatus may not be able to supply all of this demand without finally becoming exhausted.

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Relation of Certain Bile Acids to Absorption of β-Carotene in the Rat.*

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In previous work from this laboratory[1] it has been shown that deoxycholic acid acts as a carrier of irradiated ergosterol and of β-carotene across the intestinal tract of the rat. This is probably

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1 Greaves, J. D., and Schmidt, C. L. A., *J. Biol. Chem.*, 1933, **102**, 101; *Univ. of Calif. Pub. Physiol.*, 1934, **8**, 43, 49; *Am. J. Physiol.*, 1935, **111**, 492, 502; 1936, **116**, 456. Schmidt, C. L. A., *Pac. Coast Med.*, 1937, **4**, 16.

due to the formation of the respective choleic acids.[2] The present experiments were undertaken to determine whether or not taurocholic acid, glycocholic acid, and decholin can transport β-carotene in a manner similar to that of deoxycholic acid.

Two series of procedures were employed. In the first series vitamin A deficient bile fistula rats were fed a mixture of β-carotene and the bile acid under test. The vaginal smear picture was used as an index of absorption and utilization of β-carotene. Each animal received 100 γ of β-carotene daily during the test period. The activity of the carotene was controlled by feeding similar doses of the β-carotene-bile acid mixtures to unoperated vitamin A deficient rats. Thirteen bile fistula rats were employed for the glycocholic acid group, 12 for the taurocholic acid group, and 11 for the decholin group. With the exception of one rat in the glycocholic acid group none of the bile fistula rats responded to the administration of β-carotene. The vaginal smear pictures of the unoperated vitamin A deficient rats returned to normal. Under similar experimental conditions absorption of β-carotene takes place when deoxycholic acid is fed to bile fistula rats.

In the second series attempts were made to prepare compounds of β-carotene with each of the three bile acids under consideration. Ten milligram portions of β-carotene were mixed with 6 molecular equivalents of the bile acid. The mixtures were sealed in glass tubes under nitrogen and heated in a paraffin bath to 185-190° for 5 minutes. The melt was dissolved in an excess of 5% sodium carbonate solution. The filtered solutions were extracted with peroxide-free ether. Some of the solutions were previously acidified, others were not. The first part of the extract was discarded in order to remove any uncombined carotene. The remaining portions of the extracted material were evaporated to dryness in an atmosphere of nitrogen. The residue was dissolved in sodium carbonate solution and the process of extraction, evaporation, and solution was repeated. A similar experiment was carried out with deoxycholic acid.

With the exception of the deoxycholic acid-carotene preparation none of the other bile acid-carotene preparations gave a positive antimony trichloride test. The vaginal smear pictures of none of the vitamin A deficient rats, when fed the glycocholic, taurocholic, or decholin preparations, returned to normal, while a positive re-

[2] Shimizu, T., and Hatekeyama, T., *Z. physiol. Chem.*, 1929, **182**, 57; Yamasaki, K., *J. Biochem.* (Tokio), 1935, **22**, 243; Von Euler, H., and Klassman, E., *Z. physiol. Chem.*, 1933, **219**, 215.

sponse was shown by the animals which received the deoxycholic acid-carotene compound. Certain of the rats which failed to respond to the above mentioned 3 bile acid preparations showed normal vaginal smear pictures when the deoxycholic acid-carotene preparation was fed.

β-carotene, when administered intravenously either via the jugular or the portal vein, to certain animals is less effective than when it is administered by mouth.[3] The present experiments were carried out on rats. A hot saturated solution of β-carotene in acetone was poured into physiological saline solution under continuous agitation with nitrogen. The mixture was filtered and heated to remove traces of acetone. The resulting suspension was used for purposes of injection. Injections were likewise made of a suspension of a vitamin A concentrate in a gum arabic solution. This suspension was not very stable. The injections of β-carotene were made into the heart, into the carotid artery, and into the jugular and portal veins. β-carotene was also administered by means of the stomach tube and injected subcutaneously. The vitamin A suspensions were given by mouth and injections were also made into the heart and into the portal circulation. All injections were made under ether anesthesia. Doses of 0.5 to 1.0 mg. of β-carotene were administered. The doses of vitamin A were comparable.

The vaginal smear pictures of only about one-half of the rats which received injections of β-carotene into the blood stream returned to normal. The vaginal smears of these animals showed a continuous cornified cell picture within a week after the injections were made, indicating that the response to the β-carotene was of short duration. When β-carotene was injected subcutaneously most of the animals responded and the response was of somewhat longer duration than in the case of those animals in which the β-carotene was injected into the blood stream. The vaginal smears of all of the animals which received β-carotene orally became normal and continued so for at least 4 weeks.

The increase in weight of the animals which received injections of β-carotene was slight, whereas the rats which received β-carotene by mouth showed decided increases in weight. In all instances the rats which received vitamin A showed decided increases in weight and the vaginal smears returned to normal. Administration of vitamin A by mouth was found to be more effective than when the same dose was injected, although the difference was much less

[3] Rea, J. L., and Drummond, J. C., *Z. Vit. Forsch.*, 1932, **1**, 177; Drummond, J. C., and Macwalter, J., *Biochem. J.*, 1933, **27**, 1342; *J. Physiol.*, 1935, **83**, 236; Ahmad, B., Grewal, K. S., and Malik, K. S., *Ind. Med. Gaz.*, 1934, **69**, 320.

marked than in the case of β-carotene. The decreased effect of β-carotene, when injected into the blood stream, cannot be explained on the basis of passive filtration in the lungs since injections, when made directly into the portal system, were no more effective than when they were made into the heart or into the jugular vein.

Summary. No chemical evidence was obtained that taurocholic acid, glycocholic acid, and decholin form compounds with β-carotene. When β-carotene, together with taurocholic acid, glycocholic acid, or decholin, was fed to bile fistula vitamin A deficient rats, the β-carotene was not utilized. When β-carotene, in the form of a suspension, was injected intravenously, it was less effective than when administered orally. A less marked difference was noted in the case of vitamin A.

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Ultrafiltration of Psittacosis Virus.

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Levinthal[1] using the "gradocol" membranes of Elford, estimated the particle size of the virus of psittacosis to be 220 to 330mμ. Sir Henry Dale[2] has referred to unpublished experiments of Elford, in which the virus was found to be 275 mμ in diameter. No data are available on the details of filtration or the type of material used in these experiments. Microscopic measurements show the elementary body to range in size from 200 to 300 mμ,[3] while microphotographic studies give the smallest elementary bodies a diameter of 240 to 300 mμ.[4] In connection with psittacosis studies being conducted in this laboratory, it was considered of interest to determine the size of the infective particle under controlled conditions.

The virus strain used was isolated from infected shell parakeets in 1934 and has had no known connection with a human case. The virus was carried according to routine in white mice until December, 1935, when the strain was established on the chorio-allantoic mem-

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1 Levinthal, W., *Lancet*, 1935, **1**, 1207.

2 Dale, H. H., *Huxley Memorial Lecture*, 1935.

3 Lillie, R. D., *U. S. Pub. Health Rep.*, 1930, **45**, 773.

4 Coles, A. C., *Lancet*, 1930, **1**, 1011.