gogues previously studied, both in this laboratory and in others. It thus confirms the inference which we made on the basis of previous correlation studies: that visible mucus is secreted free of cells, and that the desquamation of surface columnar cells is not an integral part of the process of mucus secretion(3).

In contrast to the opacity of mucus secreted in response to the previously studied chemical mucigogues, which opacity has been found to be correlated in high degree with its columnar cell content, the mucus stimulated by ACh or related drugs is much clearer, being opalescent with a small number of more opaque fragments suspended in the mucoid mass. These fragments probably represent small particles of coagulated mucin.

In the concentrations used in the present work, ACh compares favorably with eugenol as far as rate of secretion is concerned. Thus in 65 experiments with 5% eugenol, the mean volume secreted was 6.4 cc(4), whereas in those with ACh it was 5.8 cc. But analysis of these rates is complicated by the extreme viscosity of the material secreted, with resulting slowness and irregularity of collection.

Because of its small cellular content and few gross particles, viscosimetry of native mucus proved feasible with the Ostwald technic. In this way, considerable variation in the viscosity of this ACh-stimulated mucus was observed from experiment to experiment. The meaning of such variations must await more detailed study of the chemical composition of specimens obtained by this procedure.

These studies do not furnish evidence on the mechanism of this ACh stimulation of mucus secretion, but experiments to this end are already under way.

*Summary.* The topical application of acetylcholine or related parasympathomimetic drugs to the mucosa of canine fundic pouches results in the secretion of alkaline opalescent viscid mucus which is free of columnar cells. These studies confirm our previous conclusion, that the exfoliation of gastric surface cells is not an essential part of the process of mucus secretion.

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## Plasma Cholesterol Levels of Cholesterol Fed Control and Pyridoxine Deficient Monkeys.* (18565)

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There is little information on the effects of feeding cholesterol to the rhesus monkey. Mild increases of serum cholesterol have been observed in this species by Sperry, Jailer and Engle(1) following the feeding of whole egg or egg yolks as a source of cholesterol. The present paper describes the effect of cholesterol feeding on the plasma cholesterol of pyridoxine deficient and control monkeys and was carried out in connection with our studies on the development of arteriosclerotic lesions in vitamin B6 deficient monkeys(2,3).

*Experimental.* Studies on two groups of young rhesus monkeys are reported in the present paper. The first included 8 monkeys (7 males and 1 female) weighing 1.85-2.48 kilos which were housed in individual cages. They were fed *ad libitum* the modified M-3 basal diet and the complete supplements described in a previous publication(4) for a period of 39 days. Pyridoxine was then with-

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1. Sperry, W. M., Jailer, J. W., and Engle, E. T., *Endocrinology*, 1944, v35, 38.

2. Rinehart, J. F., and Greenberg, L. D., *Am. J. Path.*, 1949, v25, 481.

3. Rinehart, J. F., and Greenberg, L. D., *Arch. Path.*, 1951, v51, 12.

TABLE I. Plasma-cholesterol Levels in Control and Pyridoxine-deficient Monkeys.

| No. and status* | Control period: Deficient period before cholesterol, days | Control period: Range, mg % | Control period: Avg, mg % | Cholesterol period: Range, mg % | Cholesterol period: Avg, mg % | Cholesterol period: Avg increase, % | Cholesterol period: Days† | Cholesterol period: Avg daily food consumption, g |
|---|---|---|---|---|---|---|---|---|
| | | | | Group I | | | | |
| 12 D | 108 | 76-131 | 103 | 124-487 | 334 | 224 | 145 | 80¶ |
| 13 D | 108 | 79-119 | 99 | 129-422 | 260 | 163 | 145 | 71 |
| 14 D | 59 | 75-100 | 87 | 165-491 | 303 | 248 | 76§ | 76 |
| 15 D | 59 | 96-103 | 99 | 156-541 | 328 | 231 | 204 | 91 |
| 19 C | — | 157-193 | 175 | 109-296 | 213 | 22 | 204 | 280 |
| 16 D | — | 92-208 | 148‡ | | ‖ | | | |
| 17 D | — | 112-237 | 174‡ | | | | | |
| 18 C | — | 81-188 | 138‡ | | | | | |
| | | | | Group II | | | | |
| 36 D | 60 | 89-192 | 98 | 220-533 | 419 | 328 | 77 | 239 |
| 37 D | 60 | 155-252 | 207 | 351-765 | 513 | 148 | 77 | 129 |
| 38 C | — | 165-219 | 182 | 263-429 | 360 | 99 | 77 | 513 |
| 35 D | — | 148-239 | 192‡ | | ‖ | | | |

* D or C (deficient or control).

† Avg in Group I include only those values obtained to time of administration of 3.5 mg doses of pyridoxine.

‡ Computed for combined intervals corresponding to control and cholesterol periods of the sterol-fed animals.

§ Sacrificed because of comatose condition resulting from marked pyridoxine deficiency.

‖ No cholesterol.

¶ Approx. value, this monkey wasted considerable amounts of food.

drawn from the diet of 6 animals, and one per cent cholesterol was added to the diet of one control and 4 deficient monkeys at the times indicated in Table I. One control and 2 deficient monkeys were allowed to remain on the basal diet without added cholesterol. The cholesterol was incorporated into the diet by merely intimately mixing the finely powdered sterol with the remaining ingredients of the diet. During the course of the experiment the plasma cholesterol levels were followed at frequent intervals for 65 weeks or longer by the method of Sackett(5). The blood used for the analyses was drawn during the early part of the morning before the day's food ration was placed in the monkeys' cages.

*Results.* The plasma cholesterol values for 3 of the monkeys, 2 deficient (nos. 12 and 13) and 1 control (no. 19) are shown graphically in Fig. 1. The ranges of the plasma cholesterol together with their average values and the average percentage increases are summarized in Table I.

The curves of the 3 monkeys in Fig. 1 are typical of the results observed in the other similarly treated members of the group and show clearly the course of the plasma cholesterol following the addition of the sterol to the diet. In the course of about 4 weeks after initiation of the cholesterol feeding, the plasma of the deficient monkeys 12 and 13 exhibited marked increases and consistently higher cholesterol levels than no. 19, the corresponding control animal. The average increase in the level of no. 19 was only 22% while during the same period the average increases in the levels of nos. 12 and 13 were 251 and 197%, with maximum values of 487 and 422 mg % respectively. The 2 other cholesterol-fed, deficient animals (nos. 14 and 15) of Group 1 attained average increases of 248 and 231%, and maximum values of 491 and 541 mg % respectively. The greater rise in the plasma cholesterol of the sterol fed deficient monkeys is all the more remarkable when one considers the fact that the average daily food consumption of these monkeys was only approximately ⅓ to ¼ that of the control animal, and hence

4. Greenberg, L. D., and Rinehart, J. F., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 20.

5. Sackett, G. E., *J. Biol. Chem.*, 1925, v64, 203.

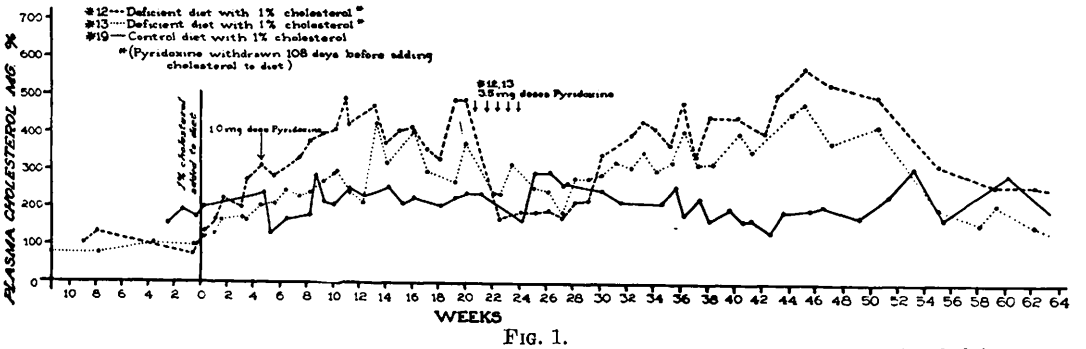


FIG. 1.

Effect of cholesterol ingestion on plasma cholesterol of control and pyridoxine-deficient monkeys.

the cholesterol intake of the latter was 3 to 4 times as great.

The administration of a single mg dose of pyridoxine hydrochloride to the deficient monkeys after 32 days of cholesterol feeding did not appear to have much influence on their levels; on the other hand, when 5 doses (3.5 mg each) of the vitamin were administered twice weekly between the 145th and 158th day of cholesterol feeding, the plasma cholesterol levels decreased soon after starting the vitamin treatment. This occurred even though the food consumption and cholesterol intake increased. The blood cholesterol levels remained low for several weeks and then returned to their former high plane within approximately 6 weeks after the last dose of pyridoxine. Without any alteration in the diet the levels again declined at the end of the 55th week. This might be accounted for to some extent by a decreased food consumption. On the other hand, Weinhouse and Hirsch(6) have made a similar observation after prolonged cholesterol feeding of rabbits and attribute the decline to impairment of absorption of cholesterol from the gastointestinal tract as a result of deposition of lipids in the lining and wall of the stomach and bowel. This explanation could be applicable in the present case.

The second group of monkeys consisted of 4 males weighing 2.01-2.28 kilos. During the course of the cholesterol studies they were fed a basal diet consisting of casein 18, powdered sucrose 59, salts 4, corn oil 2 and hydrogenated cottonseed oil (Crisco) 6. This diet differs from that fed the first group in that it contains a higher percentage of fat. The plasma cholesterol was followed before and after the introduction of 1% cholesterol into the diet. During the latter part of this experiment the deficient monkeys received daily a small suboptimal dose of pyridoxine hydrochloride (10γ per kg). The pertinent information concerning the time of introduction of the cholesterol and the nutritional status of the animals are listed in the table.

Although the second experiment has so far been carried out for a much shorter period, the results observed with the animals in Group II are essentially similar to those observed with the monkeys in Group I. The findings are summarized in Table I. The average plasma cholesterol values for the cholesterol period are higher in both deficient and control monkeys of Group II than they are for the corresponding animals of the first group. This may be accounted for by the fact that the diet of Group II contained a much higher proportion of fat and therefore probably increased the absorption of cholesterol in this group. In spite of the fact that the cholesterol ingestion was 2 to 4 times greater in the control animal his average plasma cholesterol was significantly lower than that of the deficient monkeys.

For the purpose of determining the effect of equalizing the cholesterol intake the latter was adjusted to 0.25 g of the sterol per kilo of body weight for a 6-week period. Cholesterol mixed with a small amount of sucrose and approximately 6 cc of corn oil was ad-

6. Weinhouse, S., and Hirsch, *Arch. Path.*, 1940, v30, 856.

ministered by mouth instead of being incorporated in the basal diet. The same substantial difference in the plasma cholesterol levels was encountered during this regimen. The control animal, no. 38, had an average value of 257, nos. 36† and 37†, 381 and 444 mg % respectively. As might be expected, the average plasma level of no. 38 was lowered somewhat because of a reduction in his intake of cholesterol.

*Discussion.* The greater hypercholesterolemia induced in pyridoxine deficient monkeys by the feeding of cholesterol is of interest. Whether this results from enhanced absorption or a decline in the ability of the vitamin B6 deficient monkey to handle cholesterol is not known. It is possible that cholesterol may be absorbed more efficiently in the pyridoxine-deprived animal than it is in the control. However, this appears to be contrary to what we have observed in the case of glucose. Evidence obtained in this laboratory with the latter indicates that glucose absorption is much more rapid in the animal supplied with pyridoxine. The fact that glucose absorption is decreased in the pyridoxine deficient animal does not prove that cholesterol absorption will also be retarded since they are 2 entirely unrelated chemical compounds. On the other hand, Gubler *et al.*(7) have obtained evidence which they consider indicative of increased iron absorption in the vitamin B6 deficient rat. The results with the animals in Group I do, nevertheless, show that considerable absorption of cholesterol took place in spite of the fact that the diet was low in fat, containing only 2% corn oil.

It is perhaps pertinent to note that the basal diet fed the animals is essentially cholesterol free. In spite of this, animals which have been maintained on this diet over long periods of time showed 'normal' plasma cholesterol levels indicating that cholesterol synthesis is a normal mechanism in the rhesus monkey.

While our studies are not yet completed, to date, we have not found arteriosclerotic lesions in cholesterol fed monkeys which had received adequate supplements of pyridoxine, and the arteriosclerotic lesions of pyridoxine deficiency do not appear to have been enhanced by the administration of cholesterol.

*Summary.* *Ad libitum* feeding of a low or moderate fat diet containing 1% cholesterol to rhesus monkeys resulted in a greater hypercholesterolemia in the pyridoxine deficient monkey than it did in the control monkey. This occurred despite the fact that the cholesterol intake of the control animal was 2-4 times greater than that of the deficient animals.

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7. Gubler, C. J., Cartwright, G. E., and Wintrobe, M. M., *J. Biol. Chem.*, 1949, v178, 989.

† Deficient.

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## Cortisone and Blood Pressure.* (18566)

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It is now generally accepted that the favorable effects of cortisone and the adrenocorticotropic hormone on the manifestations of many disorders are related to the production of some measure of hyperadrenocorticalism (1). The similarities of Cushing's syndrome and the alterations which ensue after cortisone therapy are noteworthy(2). With the ex-

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