Summary. 1) Repeated exposure of isolated sections of rabbit ileum to atropine (40 μg%), with washings 10 min after each exposure, results in development of tolerance, such that by sixth or seventh exposure, the depressant effects of atropine on height of spontaneous contraction are minimal or even abolished. The phenomenon of tolerance appears to be associated with gradual accumulation of atropine within the tissue. 2) Repeated exposure of rabbit ileum to adiphenine (1 mg%) results in no observable tendency toward the development of tolerance. Moreover, when adiphenine is applied to ileum which previously has been made tolerant to atropine, adiphenine is fully effective in depressing the height of spontaneous contraction.

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A Kwashiorkor-Like Syndrome Observed in Monkeys Fed Maize.* (23527)

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To understand all aspects of the pathogenesis of a particular disease in man, one should be able to reproduce it in the experimental animal. This has been eminently true in the evolution of our knowledge of certain human nutritional disease syndromes, such as beriberi, scurvy, rickets and pellagra; for great advances were made in an elucidation of the biochemical and anatomical alterations in these disease states when they could be studied in the laboratory. In contrast, our woeful ignorance of the pathogenesis of such diseases as endemic goiter and, until recently, pernicious anemia stems from the fact that the physiologic and morphologic pattern has not yet been produced in experimental animals. Today, kwashiorkor has world-wide distribution and a high prevalence. Although clinical studies, particularly those dealing with therapy, have revealed something of its pathogenesis, a more basic understanding is likely to be obtained if the disease can be reproduced in the laboratory.

Kwashiorkor is one of a group of several syndromes which occur endemically and which are associated in many parts of the world with an intake of virtually a single foodstuff. In the case of kwashiorkor this foodstuff is maize. Many years ago corn was shown to be deficient in certain inorganic elements, amino acids and vitamins(1); hence, it is not surprising that a multiple disease syndrome might follow extended use of this grain. Because investigations dealing with deficiencies of *single* essential nutrients have now furnished a baseline of biochemical and anatomical changes which may occur in many animal species, the time appears favorable to renew the study of *multiple* deficiency states such as those which may be produced by feeding single natural foodstuffs. We have recently undertaken such a program in this laboratory in an attempt to reproduce in experimental animals certain nutritional diseases which occur naturally in man and whose pathogenesis is not clear. In the present com-

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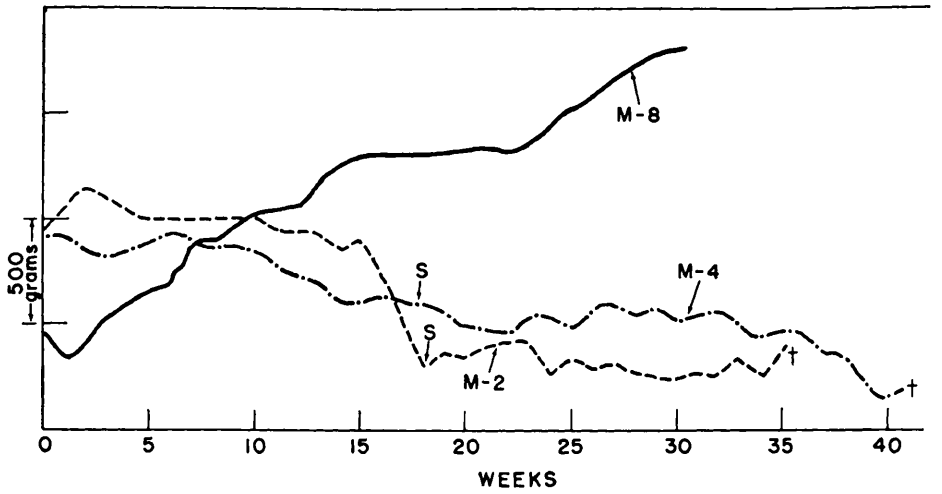

FIG. 1. Growth curves of 2 animals fed maize (M-2 and M-4) and a control (M-8).

munication we wish to describe a syndrome having many of the characteristics of kwashiorkor which has been observed to develop in monkeys fed maize.

Materials and methods. Twelve monkeys (*Cercopithecus griseoviridis* or *aethiops*) have been under study to date. All were young growing male or female specimens whose exact age was unknown, though each was in the process of acquiring its second dentition. They were housed in a well-ventilated, constant temperature, windowless room in stainless steel cages having coarse screen bottoms over cedarwood shavings. Tap water was allowed *ad libitum*. For 8, the basic foodstuff was ground unenriched yellow cornmeal (Wilkins Rogers Milling Co., Washington, D.C.) which was made into mush with water and fed raw or as cakes after heating in the oven. On analysis the corn contained per 100 g dry weight: protein, 10.2; lipid, 7.6; ash, 1.04; calcium, 55 mg; phosphorus, 215 mg; ascorbic acid,† .72 mg; thiamine,† .50 mg; riboflavin,† .18 mg; and vit. A,† 230 I.U. No other foods were given save occasional lumps of sugar and ascorbic acid, as noted below. One animal received an adequate commercial ration supplemented with sources of vit. C. Three monkeys, used in another study were fed rice supplemented with ascorbic acid and were utilized as inanition controls since their weights paralleled those of the group fed maize. Animals were fed and watered every day and weighed weekly. Blood for chemical determinations was withdrawn from the femoral vein. Liver biopsies were performed aseptically under local anesthesia with a Vim-Silverman needle. Total lipid in liver tissue was determined by ethanol-ether extraction. Complete autopsies were performed when animals died. Microscopic sections of all organs were made by routine technics. Microradiographs of ground sections of bone were made with Phillips C.M.R. equipment.

Results. After being placed on the diet a gradual loss of weight occurred. As shown in Fig. 1 for 2 representative animals, this loss was not excessive: a total of 800 and 600 g in 35 and 41 weeks per animal, when initial weights were approximately 2350 g. In contrast, the control gained 1500 g in a similar period. During the 18th week both animals shown in Fig. 1 exhibited clinical scurvy: loosening of the teeth, bleeding gums, and weakness of the lower extremities. One developed small subcutaneous hemorrhages over the surfaces of the arms and legs. Ascorbic acid (100 mg) was administered at this time, with complete amelioration of these signs. Vit. C therapy was continued at bi-weekly intervals and has been administered to all

† These analyses were performed through the courtesy of Major R. A. Huseby, M. C., Medical Nutrition Laboratory, Denver, Colo.

maize-fed animals since that time.

As the deficient state progressed, the animals became listless and apathetic. They could be handled without difficulty, making

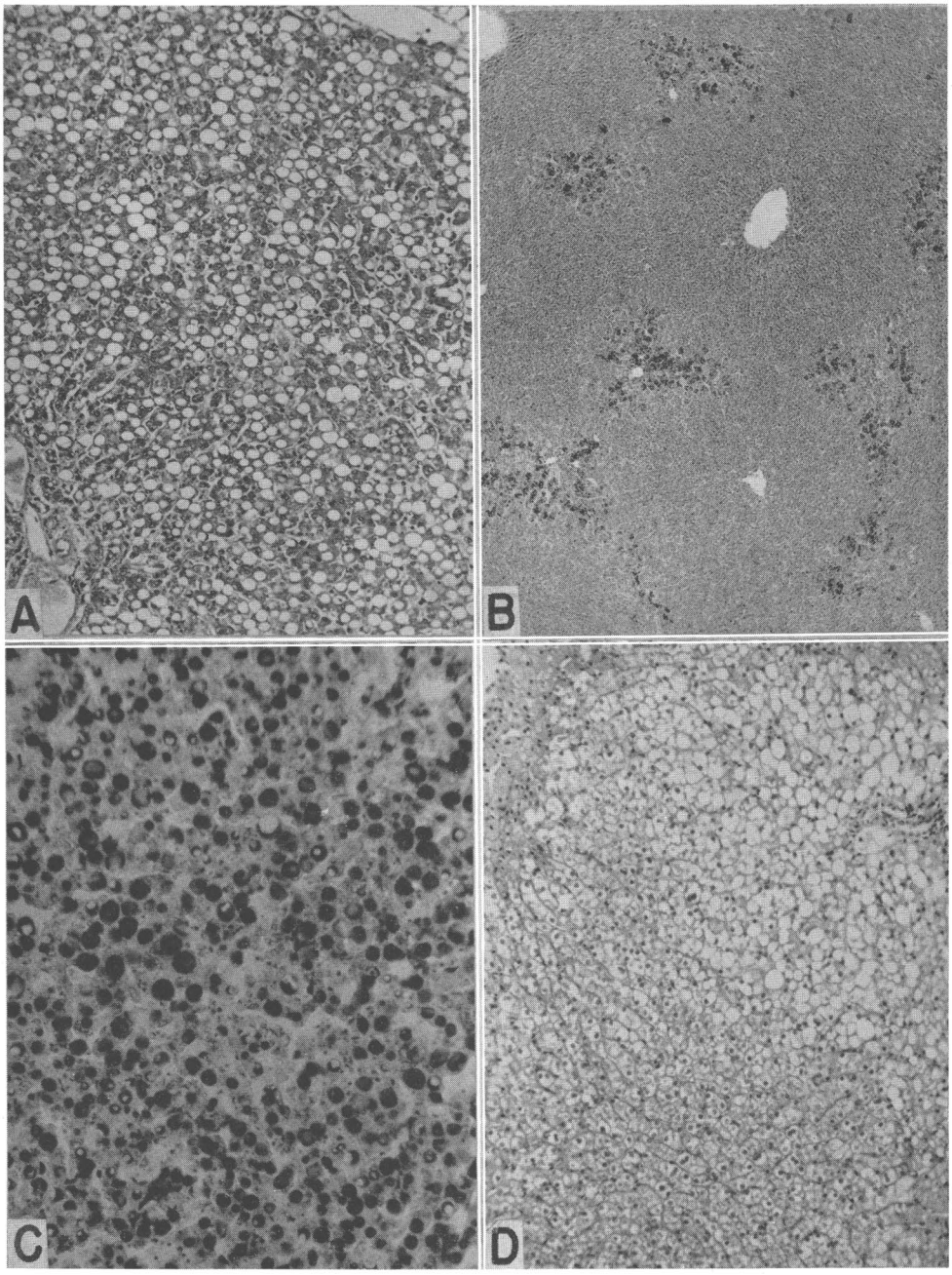

FIG. 2. A. Section from liver of monkey M-4 dying after 41 weeks on corn diet. There is extreme diffuse fatty infiltration (H. and E., × 90). B. Section from liver of animal autopsied after 13 weeks on maize diet. Fat is present only in cells of peripheral (periportal) portions of liver lobules. Note 2 central veins in this field (oil red O, × 35). C. Section from liver of animal (M-2) dying after 35 weeks to show extensive fatty change (oil red O, × 100). D. Biopsy of liver from animal (M-9) which has been on maize regimen for 14 weeks. There is periportal fatty change (H. and E., × 100).

TABLE I. Liver Lipid and Plasma Protein Concentrations.

No.	Weeks on diet	% liver lipid	Total	Alb.	Glob.
4	41 M*	22.8	3.53	.95	2.58
2	35 M	15.0	3.87	1.16	2.71
13	7 M	5.1	6.0		
8	0		6.80	4.01	2.79
12	0		7.05		
10	0		6.8		
1	0	4.2			
5	34 R*	3.9	6.9		

* M = Maize diet; R = Rice diet.

no attempt to retreat to the rear of the cage. They assumed a characteristic pose, sitting in the corner of the cage with head bent forward between front and hind limbs so that it almost touched the floor. Only when *in extremis* did the animals lie on their sides. Periorbital edema was noted in a female after 26 weeks of the deficiency. Examination at this time revealed edema about the external genitalia. Loss of hair over the top of the head was seen at the time edema became manifest in this animal. No loss of hair has been observed in other monkeys, nor has there been any change in color of the coat.

In 3 animals which have come to autopsy, the most prominent finding has been an increase of fat in the liver by chemical analysis (Table I). In the animal living longest (41 weeks) the fatty change is extreme. Less fat is present in the liver of a monkey living for 35 weeks and least in one dying after 7 weeks on the corn diet. Histological examination of the liver confirms the chemical findings. In the most severely affected livers all cells are distended with vacuoles which in frozen sections stained with oil red O prove to be lipid (Fig. 2). In such livers the distribution of fat is uniform throughout. Studies of earlier stages either at autopsy or on biopsy indicate that fat accumulation appears first in the periportal regions of the lobules and then spreads to involve the remainder of these structures. In 2 animals dying after 35 and 41 weeks on the diet iron pigment is present in large amounts within the liver cells. No inflammatory cells nor any increase in reticulum fibers have been noted.

Other alterations which have been found on microscopic examination are atrophy of intestinal glands, loss of lipids in the adrenal cortex and arrest in growth of the bones. No changes have been found in the pancreas. In the colon there is cystic dilatation of the mucosal glands whose lining cells are flattened. Cross sections of the cortices of the long bones reveal failure of the normal primary bone to be replaced by osteons (Fig. 3). Hence the skeletal tissue is more immature than it should be at this age. No evidence of osteomalacia is present.

As shown in Table I, concentrations of total plasma proteins were reduced in the 2 animals dying in the late stages of the deficiency. These monkeys had high fat concentrations in the liver. Periportal fat accumulation was beginning in the animal (M-13) sacrificed after 7 weeks, at which time the plasma protein concentration was not particularly reduced. Current biopsy studies of the liver indicate that fatty infiltration antedates changes in plasma protein concentrations.

Discussion. As it is observed in various parts of the world today, the kwashiorkor syndrome may be characterized as follows: 1) Retardation of growth, 2) Weight loss with muscular wasting, 3) Apathy and other psychic changes, 4) Dermatitis and pigmentary alterations in the hair, 5) Edema with hypoalbuminemia, 6) Enlargement of the liver as a result of fatty infiltration which begins in the periportal areas, and 7) Gastrointestinal disturbances(2). The disease is seen in children who have been weaned to a diet which is poor with respect to both quantity and quality of protein. A corn diet furnishes insufficient protein, which is deficient in at least 2 amino acids: tryptophan and lysine, and in which there may be imbalances in amino acid content as well(3). Since the early studies of McCollum(1) on the feeding of cereal grains to non-ruminants, corn has often been used in rations which were employed to study nutritional disease. For instance, the experimental production of rickets(4) in rats and blacktongue in dogs(5) depended on a high proportion of corn in the dietary regimens. Recently interest has been reawakened in corn because of its presence in large amounts in the diet in areas where kwashiorkor is endemic. Studies dealing with the inadequacies

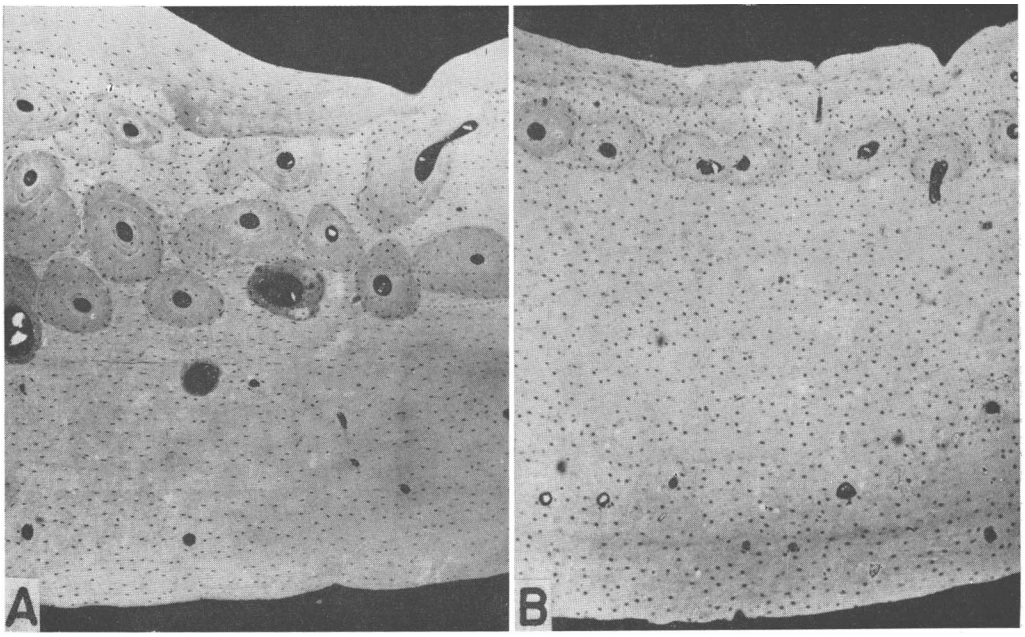

FIG. 3. A. Microradiogram of anterior cortex of tibia of normal monkey (M-8). Note numerous Haversian systems replacing the membranous bone. B. Cortex from same area of tibia of animal (M-2) dying after 35 weeks on corn. There had been no growth (see Fig. 1). Only a few osteones have appeared. There is no evidence of osteomalacia (both, × 50).

of this cereal grain, particularly with respect to protein have been reported(6,7,8,9). Such investigations have dealt primarily with growth studies in rats. However, Shils and his co-workers(8) have examined the livers of rats fed corn diets; in these animals periportal fatty infiltration has been observed. In all the studies just referred to save those of Gillman *et al.*(9) the corn rations have been supplemented by minerals and vitamins. The latter investigators utilized corn diets supplemented only with fermented milk and observed fatty livers which developed scarring. In our experience rats which have been fed a completely unsupplemented corn diet exhibit an entirely different disease syndrome(10) than that which has just been described in the monkey; hence it is felt that the rat is not a suitable species with which to work.

The experiments herein recorded have now been in progress for some time. Chemical studies, serial biopsies of animals now under observation, as well as the autopsy findings already mentioned, all appear to indicate that a syndrome rather similar to kwashiorkor can be produced in monkeys. Obviously the problem is a long term one, but in view of these promising findings it is felt this report is warranted in hopes that others may begin to study this syndrome.

Summary. Growing monkeys have been maintained up to 41 weeks on a diet of maize. Animals exhibit growth failure, loss of weight, weakness, apathy, edema with hypoalbuminemia and fat accumulation in the liver, which begins periportally and then spreads to involve the entire lobule. This syndrome appears to have many of the characteristics of kwashiorkor in children.

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Anticoagulant, Lipemia Clearing and Other Effects of Anionic Polysaccharides Extracted from Seaweed. (23528)

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Recent investigations have been made of the effects of heparin-like compounds on blood coagulation and lipemia clearance(1,2). This paper describes the hematologic and other responses to a number of such compounds. several of which have not been previously studied. These materials were prepared from aqueous extracts of red seaweeds. and are galactosans of varying degrees of sulphation.

Materials and methods. Carrageenan. a sulphated galactosan, and 6 other sulphated polysaccharides were studied.* Hyaluronate (3) and the chondroitin sulphates A(4) and B(5) were prepared using previously described methods. Measurements of lipemia were performed by reading directly the optical density of plasma in 12 x 70 mm cuvettes at 650 mμ in a Coleman Jr. spectrophotometer. Clotting times were determined in a series of small tubes ¼" in diameter containing 1 ml whole blood at 27°C. The tubes were inspected for coagulation every ½ minute. The endpoint was taken as the time when a tube could be placed on its side without any corresponding shift in blood level. Differential cell counts were done in triplicate. The hematoxylin-eosin and Hotchkiss-McManus periodic acid Schiff stains were done in the usual manner. Reducing activity was measured by a modification of the Park and Johnson procedure(6).

Twelve dogs were fed triolein (4 g/kg) after an overnight fast. The optical density of the plasma of all fasted animals was initially less than 0.09. Water was permitted *ad lib.* In 45 determinations on 12 dogs, all plasma turbidities two hours after feeding were within the range of 0.24 to 0.48 optical density unit (mean 0.41 ± 0.05). Immediately after removing 6-7 ml of blood 2 hours after the fat meal, the various polysaccharides prepared in a concentration of 2 mg/ml of saline were injected intravenously at a dose of 1 mg/kg body weight. Fifteen minutes after injection, a second blood sample was obtained. Blood was withdrawn in clean syringes and immediately added to tubes containing dry heparin. Samples of this blood were placed in clotting time tubes.

An aqueous solution of sodium heparinate was prepared containing 10 mg/ml and diluted with an equal volume of 0.3 N NaOH. After boiling this mixture for 15 minutes, the solution was cooled and 2 volumes of ethanol were added. The precipitate was removed by centrifugation and washed repeatedly with ethanol. This material demonstrated a threefold increase in reducing activity. A similar heparin solution was prepared using 6.0 N NaOH. The reducing activity increased some 15-fold and the molecule was partially desulphated. These materials were termed heparin A (normal), B (0.30 N NaOH), and C (6.0 N NaOH).

The seaweed extracts were purified by dissolving the commercial grade material in hot water and precipitating with isopropanol.

Results. The anticoagulant and lipemia clearance activities of the compounds described above were determined in triplicate.

* Seaplant Corp. of New Bedford, Mass., through the courtesy of Leonard Stoloff.