tissue were analyzed for nitrogen, complement fixing power to tissue specific (Ts) and heterogenetic antibodies (F), and phosphatase activity. The latter was determined under optimal amino acid and magnesium concentration, as described by O. Bodansky,[7] at several slightly different pH to obtain the maximum activity. Phosphatase activity $Q_{0.05}$ is the reciprocal of the time in minutes necessary to liberate 0.05 mg P per ml of solution. The phosphatase activity of each crude extract was arbitrarily considered as 100 per cent.

TABLE 1

| | (A) Kidney autolyzed for 1 week | | | | | (B) Kidney ground with sand in cold | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N/ml | Highest dilution giving complement fixation | | Phosphatase activity | | N/ml | Highest dilution giving complement fixation | | Phosphatase activity | |
| | | Ts | F | $Q_{0.05}$ | % | | Ts | F | $Q_{0.05}$ | % |
| *Experiment 1* | (2.16 gm kidney used) | | | | | | | | | |
| Crude extract | 0.71 | 0 | 0 | .0526 | 100 | 0.79 | 1/5 | 1/5 | .0167 | 100 |
| 27000 supernatant | | 0 | 0 | .0526 | 100 | | 0 | 0 | .0072 | 46 |
| % high speed sediment | 2.8 per cent. | | | | | 15.8 per cent. | | | | |
| *Experiment 2* | (1.11 gm kidney used) | | | | | | | | | |
| Crude extract | 0.60 | 0 | 0 | .074 | 100 | 0.86 | 1/9 | 1/3 | .0222 | 100 |
| 27000 supernatant | | 0 | 0 | .074 | 100 | | 0 | 0 | .0052 | 26 |
| Resuspended high speed sediment | | 0 | 0 | 0 | 0 | | 1/9 | 1/4.5 | .0164 | 82 |
| % high speed sediment | 3.3 per cent. | | | | | 12.0 per cent. | | | | |
| *Experiment 3* | (3.3 gm kidney used) | | | | | | | | | |
| Crude extract | 0.69 | 1/9 | 0 | .0394 | 100 | 0.77 | | | | |
| 27000 supernatant | | 0 | 0 | .0358 | 91 | | 1/27 | 1/3 | .0312 | 100 |
| Resuspended high speed sediment | | 1/9 | 0 | .0078 | 20 | | 0 | 0 | .0036 | 12 |
| % high speed sediment | 4.4 per cent. | | | | | 13.7 per cent. | 1/27 | 1/3 | .0264 | 85 |

Table 1 shows that in tissue extracts (B), the phosphatase activity is sedimented with the tissue specific and heterogenetic antigens. Autolysis of the tissue (A), however, causes a decrease in the amount of the heavy fraction, loss of complement fixing power to both the tissue specific and the heterogenetic antigens, with the liberation of the phosphatase in an active non-sedimentable form. Loss of complement fixing power and liberation of phosphatase from the heavy fraction seem to parallel each other. Thus in experiment 3A some tissue specific antigen remained after autolysis and a small amount of sedimentation of the phosphatase activity was found. Since some autolysis occurs even in the cold, it is not surprising that all phosphatase can not be sedimented in the non-autolyzed extracts (B). When the procedure is carried out more rapidly (within 6 hours) (experiments 2B and 3B), a much larger part of the phosphatase sediments than if carried out more slowly (over 2 days) (experiment 1B).

The association of enzymes of low molecular weight, such as phosphatase, with particles several hundred times larger in size, suggests that this heavy fraction may play an important role in the cell by acting as a carrier for certain biologically active substances. Attachment of different enzymes at various sites on this large particle would provide a structural form within the cell which may influence the sequence and direction of enzyme reactions.[8]

*Summary:* Phosphatase in extracts of mouse kidney is associated with the material sedimentable at 27,000 r.p.m. for one hour. On autolysis, this fraction decreases in amount, phosphatase is liberated, and the tissue specific and heterogenetic components are destroyed.

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[7] O. Bodansky, *Jour. Biol. Chem.*, 118: 341, 1937.

[8] I. M. Korr, Cold Spring Harbor Symposia on Quantitative Biology, 7: 74, 1939.

## THE INADEQUACY OF SYNTHETIC DIETS FOR MICE

DURING an investigation on vitamin E and experimental tumors,[1] it was found that strain A males (Bar Harbor) developed edema and protrusion of the eyeballs on a diet supposedly adequate, save for vitamin E. The basal diet (C) consisted of casein 31, corn starch 28, lard 21, salt mixture 7, cod liver oil 3 and yeast 10. Two years ago experiments were started to determine, if possible, the cause of the edema and eye condition. In the first experiment, strain A males, 4 to 5 weeks old, were placed upon diet C and divided into three groups. One group received the basal diet alone, one group received the basal diet plus the oral administration of .5 mg ascorbic acid (Eastman) three times weekly, and the final group received 30 mg of a tested vitamin E concentrate every two weeks. Ten mgs of this E concentrate would insure pregnancy in E deficient strain A females. The growth and physical appearance of the mice were excellent until the mice reached the age of 8–12 months. At this time the following symptoms occurred: sore eyes, uni- or bilateral, characterized by swelling and inflammation of the eyelids leading to closure of the eyes and blindness

[1] C. Carruthers, *Am. Jour. Cancer*, 35: 546.

in many cases, dermatitis on the ventral aspect of the neck between the forelegs and extending up almost to the lower lip, and some loss of hair especially on the face and neck. All these symptoms were followed by death.

On diet C the females showed the same symptoms about one month later than the males. However, the former showed the same phenomena in a large number of the mice as early as 7 months on the following diet (D): casein 31, sucrose 28, rancid lard 21, salt mixture 7, cod liver oil 3 and yeast 10 or 15. The cod liver oil was mixed with the other constituents just before feeding. The oral administration of cod liver oil three times weekly or of hydroquinone 5 mg three times weekly from the time of weaning failed to prevent the occurrence of the symptoms. Those receiving a tested vitamin E concentrate showed the disease somewhat later.

The following changes in diet C have not resulted in curing the symptoms nor have they prevented them when instituted at the time of weaning (the changes were made at the expense of the starch, and the diets were stored at 0° C.): yeast 15% and cod liver oil 5%; yeast 15%, cod liver oil 5% and casein 18%; yeast 15%, cod liver oil 5% and salt mixture 8%, 7% and 4%; in the female yeast 10% and cod liver oil 5%. The following substances have been ineffective in preventing the fatal outcome: vitamin A concentrate in the natural ester form, cod liver oil, ether extracted wheat germ oil, tested vitamin E concentrate, vitamins $B_1$ and $B_6$, riboflavin, nicotinic acid (.5% in diet C), alfalfa meal (10% and 20% in diet C), choline, lemon juice (entire lemon) and tomato juice. Mice were placed on the modified diets or given the various supplements when the deficiency was manifest by swelling and inflammation of the eyelids.

Purina Dog Chow and Rockland Mouse pellets prevented death and brought about apparent cure in most of the deficient mice in 4 to 6 weeks. A small percentage was resistant. Many natural foodstuffs have been incorporated into diet C at 10% and 20% levels and, up to now, fresh flaked wheat germ and aqueous liver extract were found to have the most potent curative properties. But under the experimental conditions observed by us so far, it has not been possible to obtain a 100% recovery with these two materials. A percentage varying from 40% to 60% died or was not completely cured, thus indicating either that the damage, if too extensive, is irreparable, or that the substances now tested are not the most potent. These observations would seem to indicate that a real deficiency exists for quite some time before it becomes apparent, thus making complete cure difficult. The substances showing curative properties, and others are now being tested both as preventives and curatives.

This work indicates a true nutritional deficiency disease for mice, since the symptoms are not observed in mice on stock diets nor do rats show these symptoms on similar diets. Over 1,100 mice have been used in these experiments and the regularity of the symptoms followed by the fatal outcome, unless the mice are removed from the synthetic diets, is further evidence that a deficiency exists. New Buffalo (Simpson) and Old Buffalo mice show the same symptoms on the same diets. Recently Woolley[2, 3] has shown that inositol or its derivatives are required by the mouse for normal growth and hair. Also Norris and Hauschildt[4] have found that the mouse requires a water soluble fraction of the vitamin B complex for growth and healthy skin.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## AN APPARATUS FOR MEASURING MICROSCOPIC OBJECTS

IN studies on bovine anaplasmosis, it was found desirable to measure the anaplasms or marginal bodies occurring in the erythrocytes of infected cattle. These bodies measure less than 1 micron in diameter and an ordinary ocular micrometer disc is not divided into sufficiently small units to give the accuracy needed. Difficulty was experienced in using a camera-lucida and the ordinary scale drawn on paper because the markings were so close together that it was necessary to use intense illumination on the scale in order to see the rulings in the camera-lucida. As a result, the limits of the image of the object to be measured were not well defined.

In order to avoid these difficulties and measure in tenths of a micron with reasonable accuracy, an apparatus (Fig. 1) was devised to replace the ordinary measuring scales. A microscope was fitted with a monocular tube, an oil immersion lens, a 15× ocular, and a camera-lucida from which the mirror had been removed. A box 2 feet square and 1 foot deep was fitted with 4 electric lights and placed about 10 feet away from the microscope in a lateral direction. The open side of the box was turned toward the opening in the side of the camera-lucida and was covered by a large piece of cardboard in which the scale had been

[2] D. W. Woolley, *Jour. Biol. Chem.*, 136: 113.
[3] D. W. Woolley, SCIENCE, 92: 384.
[4] E. R. Norris and J. Hauschildt, SCIENCE, 92: 316.