

cold alcohol. The salt mixture was that employed by Osborne and Mendel. Thirty-five rats in all were used for this experiment. They were kept on this rigid diet for a period averaging about three to four months, but extending in several cases five to six months. In no instance were rachitic lesions noted either microscopically or macroscopically, merely an inactive osteogenesis. That the dietary in point of fact did contain only a minimal amount of the fat-soluble vitamine was proved by the lack of gain of the animals after they had been on this food mixture for about sixty days, by their prompt response in growth on the addition of 6 per cent. of butter fat to the diet, and by the development of ophthalmia or keratomalacia in almost all of the animals and its rapid subsidence on adding butter to the dietary. In addition to eye lesions the rats on a restricted diet developed many infections. Those receiving 0.5 c.c. of orange juice did not, however, develop either ophthalmia or infections as frequently as others where the diet did not include this food. Ten control animals which received the same diet, with, however, an addition of butter, did not develop ophthalmia, grew normally and remained in perfect health. Our deduction from these experiments is that a lack of the fat-soluble vitamine in a dietary which is otherwise complete does not lead to the development of rachitic lesions in rats.

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**The effect of various modifications of a diet producing rickets
in rats.**

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At the March meeting of this Society, it was shown by Sherman and by one of the writers of this paper that rickets regularly developed in rats maintained on a diet composed of patent flour, calcium lactate, sodium chloride and ferric citrate. It was further found that the substitution of 0.4 per cent. basic potassium

phosphate for an equal percentage of calcium lactate in this diet (No. 84), uniformly protected against the development of rickets.

These experiments have been continued. The diet has been modified in various ways, and we wish this evening to report very briefly some of the results obtained.

The basic rickets-producing diet has been tested on a further series of rats, amongst them controls for other experiments. Including the 15 rats referred to in the previous paper, 36 rats in all have been observed to develop rickets upon Diet 84. A few of these after having shown unmistakable rachitic changes by x-ray, were subsequently given other diets. In all the other rats, the diagnosis has been confirmed by microscopic examination. It may be confidently stated then that rickets will develop in 100 per cent. of animals upon this diet; at least, in our experience, there have been no exceptions.

The protective action of the basic potassium phosphate has also been demonstrated in 9 additional rats; and there have been no failures amongst the total 24 rats, which have been studied up to date.

The first question to be answered was as to the part played by the potassium and the phosphate respectively, in the protection afforded by the basic potassium phosphate. To determine this point, an equivalent amount of primary *sodium* phosphate was substituted for the potassium salt in the diet; and in another series of the same litter, an equivalent amount of potassium *chloride* for the potassium phosphate. The results of this experiment are shown in Table II, from which it is apparent that the protection is conferred by the *phosphate*, and not by the *potassium*.

Experiments to determine the minimal amount of phosphate (calculated as P), which, when added to the basic diet 84 will afford complete protection, are not yet completed. The data given, however, indicate that this limit lies between 50 and 25 mg. per cent., the original diet 85, containing 72 mg. of added phosphorus per 100 gms. of diet, in addition to the 87 mg. contained in the basic diet 84.

As was pointed out in the previous paper, Diet 84 is inadequate for proper growth, being deficient not only as to the character of

the protein, but in both fat soluble A, and possibly in its water soluble B vitamins. The experiments summarized in Table IV show the effect of the addition of various substances to the rickets-producing diet.

1. Four rats receiving an addition of 0.2 gm. of pasteurized butter daily, developed rickets, the lesions being quite as severe as those found in the control rats of the same litter maintained on Diet 84 without butter. Further experiments are in progress, using fresh *unpasteurized* butter. However, there is good reason to believe that the butter used was adequate as regards its content of fat soluble A. Ophthalmia did not occur in the butter-fed rats, but was found in approximately 90 per cent. of the rats on Diet 84 alone. Furthermore, a typical gain in weight was obtained when this pasteurized butter was added to a fat-soluble vitamin deficient diet.

This experiment, we believe, adds further confirmation to the data presented by Hess and his co-workers. In rats, absence of fat soluble vitamin in the diet does not produce rickets, nor does its presence prevent it.

2. The addition of 60 mg. of "Harris yeast vitamin" daily to diet 84 gave complete protection in 3 rats. The phosphorus content of this yeast preparation is so high that it comes within the range which, in the form of an equivalent amount of phosphate, would confer protection. Nothing definite can, therefore, be deduced from this experiment, as to the possible protection attributable to the water-soluble vitamin factor. It is interesting to note that all three rats of this series showed ophthalmia.

3. The addition of 10 gms. of purified casein to 100 gms. of Diet 84 (containing phosphorus equivalent to the 72 mg. present in Diet 85), gave results which are difficult to interpret. All three rats of this series showed in the x-rays taken after 22 days on the diet, distinct rachitic lesions. One of the rats, sacrificed on this day, had definite rickets, grossly and microscopically. The other two were allowed to continue to the 38th day, the x-rays taken at death, showing an apparent healing of the rachitic lesions in the head of the tibia. The microscopic study of the ribs in these rats shows no active rickets. Further experiments are planned to compare quantitatively the protection given by casein with that given by inorganic phosphate.

4. The addition of both casein and yeast greatly improved the growth and nutrition of the rats. As was to be expected from the results with the addition of yeast alone, there was complete protection afforded.

The experiments here reported must be regarded as preliminary to more detailed studies. The ease and certainty with which the disease can be produced in rats cannot but make its experimental study profitable, although it would be obviously premature to apply the data already obtained to the problem of human rickets.

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**Diffusible calcium in normal, rachitic, and
experimental tetany blood.**

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In 1911, Rona and Takahashi¹ reported their work on the diffusible Ca of horse, ox, and pig serum, finding an average of 65 per cent. of the total serum Ca to be diffusible. No work was done with human blood. MacCallum, Lambert and Vogel² in 1914 made the following statement: "If tetany blood be dialyzed under exactly the same conditions as normal blood, it still loses a proportionate amount of its Ca, which would perhaps show that it is not especially the loss of a diffusible Ca as contrasted with a non-diffusible form—which is important in producing tetany." Brinkman³ in 1919 advanced the hypothesis that the calcium-ion concentration is dependent on the CO₂ tension of the blood. In view of the altered blood CO₂ combining power found in tetany by some workers, we have endeavored to correlate Brinkman's hypothesis with the low Ca content of the blood in tetany. During

¹ Rona and Takahashi, *Biochem. Ztschr.*, 1911, xxxi, 336.

² MacCallum, Lambert and Vogel, *Jour. Exp. Med.*, 1914, xx, 149.

³ Brinkman, *Biochem. Ztschr.*, 1919, xlv, 101.