

to recover *Bacterium granulosus* in pure culture by the usual methods of diluting and plating.

In order to overcome this difficulty we have attempted to separate the smaller from the larger organisms by means of differential filtration.

Berkefeld "V" filters, new or used, are selected and tested for permeability for *B. prodigiosus* and staphylococci. The filters which allow the passage of *B. prodigiosus* but not of staphylococci are chosen. A suspension of the contaminated material is diluted in normal saline solution and passed through the filter. The filtrate is either spread on plates or inoculated into leptospira medium. Occasionally other small bacteria may be found in the cultures of the filtrate along with *Bacterium granulosus*. These other organ-

isms have not offered the same technical difficulty of separation from *Bacterium granulosus* as have staphylococci, diphtheroids and other large bacteria which rapidly overgrow and suppress it.

This method, therefore, has been satisfactory in separating *Bacterium granulosus* from contaminated cultures in which it has been difficult or impossible to recover the organism in the usual manner.

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SPECIAL ARTICLES

A RATION FOR THE PRODUCTION OF RICKETS IN CHICKS

Up to the present time we have used a ration consisting of 97 parts of ground yellow corn, 2 parts of calcium carbonate, 1 part of common salt, and skimmed milk *ad libitum* for the production of rickets in growing chicks. The ration is extremely low in vitamin D, and when day-old chicks are placed on the ration severe rickets is produced in 5 to 6 weeks. The ash content of the extracted tibia removed from these chicks is about 30 to 33 per cent., while the ash content of similarly treated tibia from normal White Leghorn chicks of the same age is about 40 to 45 per cent. or often somewhat higher. The addition of various sources of vitamin D to this ration, together with a study of the ash content of the bone, furnishes an excellent method for the determination of the vitamin D potency of these materials. However in the evaluation of sources of vitamin D, especially cod liver oils and other fish oils, for the poultry industry as well as for the scientific study of rickets in chicks, there has been a demand for a suitable dry ration. State inspection laboratories, looking forward to the control of marketable sources of vitamin D, find it inconvenient to use a ration containing liquid skimmed milk because of the difficulty of procuring skimmed milk daily. Another objection to the liquid skimmed milk ration is the variable intake that follows its use. Consequently we have, during the past two years, attempted to develop a suitable dry ration for the study of rickets in chicks. Dry rations have been used in several commercial laboratories and in institutions studying the problem of rickets in chicks; but it would seem advisable if the ration here proposed or some other equally suitable ration could be more generally adopted.

A ration to be satisfactory must be palatable, give good growth and in the presence of vitamin D contain

sufficient calcium and phosphorus in the proper ratio for optimum calcification of the skeleton. We believe, too, that the ration should be one that gives pronounced rickets in 4 to 5 weeks—in fact, may lead to a high mortality at that age. The chick itself is particularly suitable for studies of bone development because of its wonderful sensitiveness to the absence of vitamin D. One does not need a one-sided ration, that is, high calcium and low phosphorus, or high phosphorus and low calcium, to produce rickets in the chick as has been commonly used in the production of rickets in the rat. In the case of the chick severe rickets is readily produced where the calcium and phosphorus content of the ration is at a reasonable level, and with an optimum ratio, such as 2 parts of calcium to 1 part of phosphorus.

It is true with the chick, as with other animals, that the mineralization of the bones can be influenced by the level and ratio of calcium and phosphorus in the ration. With the use of what is known as the Wisconsin baby chick ration, in which the calcium and phosphorus are particularly high, that is, approximately 2.5 per cent. of calcium and .7 per cent. of phosphorus, rickets is delayed in the absence of vitamin D and fair mineralization of the bones results during the early growing period. On the other hand, in the ration that we are suggesting for a standard rachitic ration with chicks, the calcium and phosphorus are sufficiently high to give excellent calcification where vitamin D is adequately supplied, but a very distinct picture of rickets in the absence of vitamin D. The ration that we have finally adopted is one consisting of:

59 parts of ground yellow corn
25 parts of wheat middlings (standard)
12 parts of crude casein
1 part of common salt

- 1 part of precipitated calcium carbonate
- 1 part of precipitated calcium phosphate
- 1 part dried yeast¹

This ration contains from 19 to 20 per cent. of protein, .9 to 1 per cent. of calcium, and .5 to .6 per cent. of phosphorus. It is composed of materials that are readily obtainable for any laboratory and of standard materials available on the market. The yeast was added because of the better growth that was obtained through its use. White Leghorn chicks started on this ration in the presence of an adequate supply of vitamin D will weigh 300 to 325 grams at the end of 6 weeks; without the yeast they will be 50 to 100 grams lighter. We preferred to have a ration that gave good rates of growth. Day-old White Leghorn chicks placed upon this ration will become distinctly rachitic in from 28 to 35 days, and some of the birds may be dead by the end of the fifth week, with an ash content of the extracted tibia approximating 30 per cent. In the presence of an adequate supply of vitamin D, White Leghorn chicks fed this experimental ration for 35 days will weigh about 225 grams, and the ash content of the extracted tibia will generally be 43+ per cent.

Our technique in conducting the experiment is as follows: White Leghorn chicks, one-day old and weighing 30 to 35 grams, are placed in groups of 6 or 8 in warmed hovers provided with screen bottoms. These screen bottoms are made of wire mesh, either two or three mesh to the inch, and used for the purpose of minimizing excreta consumption. Shavings are placed under this false screen bottom. The birds are fed water only for the first day; but on the second day they are given small portions of the ration upon a cardboard mat. On the third day the ration is placed in suitable feeders and so continued during the 5 weeks of the experiment. If consumption records are desired the birds can be placed in individual cages and fed through suitable grids, whereby accurate consumption records can be obtained. The birds are weighed weekly. At the end of the fifth week they are killed, the tibiae removed and placed in 95 per cent. alcohol until convenient to proceed with the analysis. They are then crushed, individually wrapped in filter-paper, and extracted for 72 hours with hot 95 per cent. alcohol. Finally the bones are dried, weighed, and ashed in an electric muffle furnace for 1 hour at a cherry red heat (about 650° C.). The percentage of ash is used as the index for estimating the degree of calcification.

The curative type of experiment and the "line test" as used in the case of the rat are not possible in the

case of the chick. In the healing of rickets in the chick there is no distinct line of calcification, but healing is diaphyseal and immediately contiguous with the trabeculae that remain after rickets is produced. Consequently no distinctive "line" is formed during healing.

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AN INVESTIGATION OF "INSIGHT" IN RATS

IN some preliminary experiments conducted recently the writer sought to get further evidence on the existence of "insight" in rats.

The problem was formulated as follows: If rats have learned to take a difficult path to food in preference to an easy but blocked path, and if then the easy path is offered as a short cut to food, what type of reactions will occur? According to a mechanistic theory of learning the rats should continue to take the difficult path even if the easy path has been so arranged as to lead to food under the most favorable conditions. On the other hand according to theories which oppose the simple mechanistic formulations, one might expect the operation of "insight" as indicated by an immediate or rapid switch to the easier path.

The experimental setting designed to meet the conditions of the problem was as follows: An elevated, open maze was used.¹ (See Fig. 1.) The easy path W was an open running path blocked at B near the

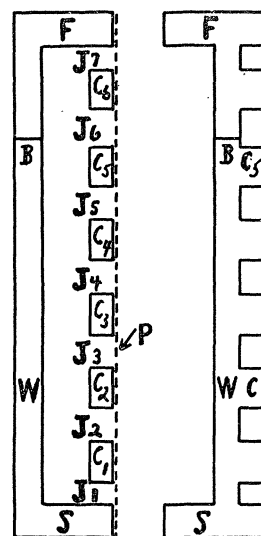


FIG. 1

FIG. 2

¹ The yeast was obtained from the Northwestern Yeast Company, Chicago, Illinois. Experimental yeast (powdered yeast foam tablets) with 50 per cent. + of protein).

¹ W. R. Miles, "The Narrow Path Elevated Maze," *Proc. Soc. Exp. Biol. and Med.*, 24: 454-456, 1927.