TABLE 1

	Ration 604	Ration 610
(Dried beef kidney 15)		
(Dextrin ..... 48)	71	69
Crude casein	14	14
Brewer's yeast	2	2
Salts I	5	5
$Ca_3(PO_4)_2$	3	5
Alcoholic extract rice bran ..	5	5
Percomorph oil—3 drops twice weekly		

claved rice bran failed to protect. The ash of rice bran or the manganese equivalent to that of 15 or 20 per cent. of rice bran also failed to protect when fed with ration 604.

These observations led to an investigation of the phosphorus distribution in the blood of normal and perosis birds as well as the phosphatase content of the blood and bone. It was found that the inorganic phosphorus of the blood remained constant in both normal and slipped tendon birds and at a level of approximately 4.7–5.6 mg per 100 cc of blood. The ester phosphorus was approximately 26–30 mg per 100 cc of blood in the case of perosis, while the total phosphorus ranged from 100 to 141 mg per 100 cc of blood. In normal birds produced by feeding or injecting manganese, the ester phosphorus ranged from 32–44 mg per 100 cc of blood, while the total phosphorus varied from 100–132 mg per 100 cc of blood. The most characteristic feature of the phosphorus distribution in the normal and afflicted birds was a higher ester phosphate in the blood of the normal birds.

In respect to the phosphatase content of bone and blood of normal and slipped tendon birds, there was also a clear-cut difference. On ration 604—which produced 100 per cent. slipped tendon—the phosphatase content of the blood ranged from 2.1–3.1 units per 100 cc of blood and from 3.6–7.7 units per gram of green bone. In the birds protected by manganese feeding or injection at different levels, the phosphatase content of the blood varied from 15.9–51.3 units per 100 cc of blood and from 8.5–10 units per gram of green bone. It is apparent that in the complex process of normal bone formation, a high inorganic calcium phosphate ingestion had depressed the phosphatase content of blood and bone, and at the same time there had occurred a lowering in the ester phosphate level of the blood.

It is possible that the autoclaving of rice bran, which is then rendered ineffective as a protective agent, is linked with a destruction of the phosphatases of the bran. Since rice bran is rich in phytin—the calcium-magnesium salt of phytic acid—we raised the question as to whether there was a possibility that inositol (a constituent of phytic acid) might be concerned in the ester phosphate increase observed in normal birds as compared with those afflicted with slipped tendon. Feeding inositol on ration 604 at a level of 5 grams per kilo did not protect against perosis. Injection of inositol at the rate of 50 mg per week did not protect with ration 604. However, we have observed that with ration 610—containing 5 per cent. of calcium phosphate—and supplemented with 20 mg of manganese per kilo, there is no protection against perosis. The manganese level is not high enough. The ester phosphorus remains below 30 mg per 100 cc of blood and the phosphatase at 30 units per 100 cc of blood and 6.4 units per gram of green bone. When the chicks receiving ration 610 supplemented with 20 mg of manganese per kilo were injected with 100 mg of inositol per week, there was complete protection against perosis. The ester phosphorus rose to 34 mg per 100 cc of blood and the phosphatases of the blood to 40.5 units per 100 cc. With the same ration injection of 10 mg of inositol or of 100 mg of glucose per week did not protect. We hesitate at this time to state definitely that the increase in ester phosphorus of the blood by injection of adequate manganese or inadequate manganese plus inositol resulted in the formation of inositol esters of phosphoric acid, but such a possibility may well exist. So far as we know, no one has isolated from animal tissue phosphoric esters of inositol, although they are known to exist abundantly in the seeds of certain plants. Free inositol itself is known to occur in muscle and brain; however, its function has not been disclosed.

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THE GROWTH OF LEPTOSPIRA ICTERO-HEMORRHAGIAE ON THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO

A WIDE variety of bacteria and viruses has been successfully grown in the tissues of the chorio-allantoic membrane of the chick embryo by a number of workers.[1] Up to the present time, however, no one has described the cultivation by this method of any member of the family *Spirochetales*. The purpose of the present note is to report the use of chorio-allantois of the chick embryo for the successful cultivation of *Leptospira icterohemorrhagiae,* the causative organism of Weil's disease (infectious spirochetal jaundice or *spirochetosis icterohemorrhagica*).

The strain of *Leptospira icterohemorrhagiae* employed was isolated at autopsy from the kidney of a man who died of Weil's disease at Rochester, N. Y., on February 19, 1937. The strain was maintained by passage in guinea pigs and by cultivation in Fletcher's

[1] See reviews by F. M. Burnett, *Med. Res. Council, Sp. Rept. Series,* No. 220, 1936; also, E. W. Goodpasture, *Amer. Jour. Hyg.,* 28: 111, 1938.

medium.[2] On May 5, 1938, 0.1 cc of a positive culture was inoculated on the chorio-allantoic membrane of each of 10 eggs, incubated for 10 days, according to the method of Goodpasture and Buddingh.[3] Transfers were made every 4 or 5 days. To effect a transfer, 2 or 3 membranes were triturated in a mortar with Alundum and Locke's solution to make a 10 per cent. suspension. For the inoculation of each egg, 0.1 cc of this preparation was dropped on the presenting ectodermal surface of the chorio-allantois.

The spirochetal organisms were carried through 20 successive passages in the developing egg. After each fifth passage, 1 cc of the "transfer inoculum"—the 10 per cent. suspension of triturated infected membranes—was injected subcutaneously into each of 2 guinea pigs. In every instance, the inoculated guinea pig developed fever within 4 or 5 days and became jaundiced. The infection was uniformly fatal in from 6 to 8 days. At autopsy, the characteristic findings of experimental Weil's disease in guinea pigs were encountered. Jaundice of the skin, jaundice and hemorrhages in the subcutaneous and cartilaginous tissues and hemorrhages in the lungs and abdominal viscera were typical.

The organisms were seen by the darkfield technic in centrifugalized "transfer inoculum" and in the amniotic fluid.

After inoculation on the chorio-allantois, the organisms regularly invaded the embryo. The resulting generalized infection killed the embryos in 6 or 7 days. The organisms have been recovered from the blood of the embryos and from various tissues by the inoculation of these materials into guinea pigs. It has been found that 0.15 cc of blood from the allantoic artery contain sufficient spirochetes to kill guinea pigs within the usual period.

Grossly, the membranes showed scattered, grayish, opaque, pin-point nodules. Microscopically, the nodules were seen to be the result of a localized proliferation of ectodermal cells, and of edema, proliferation of the fibroblasts and infiltration of a few inflammatory cells in the mesoderm. Sections of infected chorio-allantoic membranes and embryos, stained according to the silver impregnation technic of Levaditi, revealed the presence of *Leptospira icterohemorrhagiae* in both the membranes and embryos.

In summary, it has been shown that *Leptospira icterohemorrhagiae* can be successfully cultivated in the chorio-allantoic membrane of the chick embryo. The organisms produced a generalized and fatal infection in the developing chick. After 20 serial passages in the embryonic tissues, the pathogenicity of the spirochetes for guinea pigs was unaltered. Indeed, virulence may have been enhanced. An attempt is being made to grow other members of the family *Spirochetales* by this method.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN IMPROVED APPARATUS FOR THE SERIAL SECTIONING OF FOSSILS

IN 1933 Simpson (*Amer. Mus. Nov.* No. 634) described a very simple method of producing serial sections of fossils to which the reader is referred for the actual technique. Without claiming to introduce something essentially new it is suggested that the apparatus then described is capable of certain improvements, making it less delicate to handle, and at the same time increasing its accuracy. The number of components is even smaller, and the actual execution is still within the scope of any mechanic provided with an ordinary instrument-maker's lathe. The overall length of the new design is greater than that of the original construction, which may even be an advantage affording a better grip. This greater length is due to the holder being extended to carry a millimeter scale above the threaded portion, the pitch of the thread being one mm. The sleeve on its conical upper end is divided into 100 parts, the two scales together forming a micrometer reading to 0.01 mm. This arrangement prevents the locknut being placed above the sleeve. Instead it has been placed inside the latter and is made in one piece with the guide. Its lower edge stands 0.5 mm behind the lower edge of the sleeve. A suitable spanner with two studs fitting the two holes in the lower rim of the guide (locknut) is used for tightening it. If a right-hand thread is used the scales should read in the direction given in the diagram.

The advantages claimed are the following: absence of the delicate pointer, which is easily bent; the threads are covered and thus protected from injury; the scale is an integral part of the sleeve, doing away with the sticking on of a paper-scale; mistakes in taking the readings are less to be feared.

In order to reduce wear of the lower edge of the sleeve a ring could be shrunk on to it, either of hardened steel, or better still, of one of the modern cutting metals like Stellite, Acrite, etc. In this case it might be necessary to use kerosene oil instead of water for

[2] W. Fletcher, *Trans. Roy. Soc. Trop. Med.*, 21: 265, 1928.

[3] E. W. Goodpasture and G. J. Buddingh, *Amer. Jour. Hyg.*, 21: 319, 1935.