32½% citrate. Recovery is very apparent although the albumin-globulin ratio is even less than that shown in Fig. 3.

The right hand curve in Fig. 4, made by precipitation of the serum 3 months before the acute infection, indicates that there had been a still greater amount of albumin as well as a greater albumin-globulin ratio at that time.

That the curve in Fig. 3 is not peculiar to one case may be seen from the left hand curve which represents another similar case during an acute infection.

Conclusions. There are 3 obvious changes in a salting out curve of serum of patients in the degenerative states of Bright's disease: (1) Decreased englobulin or absence of it. (2) Decreased albumin. (3) Presence of a fraction not occurring in normal serum. These changes are particularly pronounced where there is an acute infection. They can not be observed by making an albumin-globulin ratio.

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Importance of the Liver for the Antirachitic Efficacy of Vitamin D.

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The mode of action of vitamin D is at present unknown. A new hypothesis was suggested by Gerstenberger[1] which, on the basis of clinical observations, considered that the action of vitamin D was concerned with some function of the liver thus far unknown. The purpose of the investigation reported here was to approach this hypothesis in animal experiments, in order (1) to study the antirachitic efficacy of vitamin D in rachitic rats in which obstructive biliary cirrhosis had been induced or in which liver damage had been caused by the administration of carbon tetrachloride and (2) to learn whether the resulting decreased antirachitic potency of vitamin D was due to the injury to the liver itself or to other circumstances accompanying the experimental conditions.

Steenbock's rachitogenic Ration 2965 was given to rats 3 weeks of age. To induce cirrhosis of the liver, double ligation and transection of the common bile duct was performed 1 to 1½ weeks after

[1] Gerstenberger, H. J., *Monatschr. f. Kinderh.*, 1933, **56**, 217.

the diet had been started. To induce liver damage, 0.2 to 0.3 cc. of carbon tetrachloride was given by intramuscular injection every second day for 3 consecutive weeks, starting also 1 to 1½ weeks after the rats were placed on the Steenbock ration.

After 3 weeks of the rickets-producing diet (that is, 1½ weeks after operation or after beginning of administration of carbon tetrachloride), antirachitic treatment was begun by intramuscular injection of viosterol in oil, Drisdol, or sodium glycerophosphate solution daily for ten consecutive days.

Under these conditions it was found that bile stasis led regularly in 3 weeks to severe obstructive biliary cirrhosis and that the parenteral administration of carbon tetrachloride resulted in severe fatty and parenchymatous degeneration of the liver rather than in cirrhosis. The results, summarized in Table I, show that 10 to 12 times

TABLE I.
Curative Doses of Vitamin D and Glycerophosphate Solution in Rachitic Rats with and without Biliary Cirrhosis or Liver Damage.

Groups of rachitic rats used	Vitamin D given by intramuscular injection as: Viosterol in oil, U.S.P. units	Viosterol in oil, No. of rats	Drisdol, U.S.P. units	Drisdol, No. of rats	Glycerophosphate solution (10%) given by intramuscular injection, cc.	Glycerophosphate solution (10%) given by intramuscular injection, No. of rats
Without biliary cirrhosis or liver damage (controls)	2.5	22	<2.5	14	0.2	11
With biliary cirrhosis	25-30	34	30	44	0.15	11
With liver damage produced by administration of carbon tetrachloride	5-8	63	—	—	—	—

as much vitamin D (given as viosterol in oil) was needed to cure rickets in rachitic rats having obstructive biliary cirrhosis as was needed in the rachitic controls. This finding confirms similar observations by Greaves and Schmidt.[2] Their interpretation, however, was that the decreased curative efficacy of vitamin D in jaundiced rachitic rats was due (1) to the lack of intestinal absorption of vitamin D caused by absence of bile in the chyme and (2) to a functional impairment of the osteogenic cells caused by the jaundice.

All the curative agents in the author's experiments were given by intramuscular injection; therefore a lack of intestinal absorption

[2] Greaves, J. D., and Schmidt, C. L. A., *Univ. California Pub. Physiol.*, 1934, **8**, 49.

does not have to be considered. Furthermore, it was found (Table I) that an even less amount of phosphate solution was needed to produce calcification in the epiphyses of the rats in the jaundiced group than of the controls. It consequently can be concluded that jaundice does not lead to an impairment of the calcifying function of the osteogenic cells. This conclusion is also evident from the results obtained when viosterol was administered to the carbon tetrachloride group in which jaundice, if it was present at all, developed only to a mild degree. That the curative potency of vitamin D is not so markedly diminished in the carbon tetrachloride group as in the rats with cirrhosis probably has to do with the very different kind of pathology produced in the liver by the two methods. In view of the facts that the rats injured by bile stasis or by carbon tetrachloride gained less weight than did the controls and that inhibited growth aids rather than interferes with healing of rickets, it may be said that the reduction in curative effectiveness of the antirachitic agents used would have been even greater if the rats with cirrhosis or those with liver damage had had the same rate of growth as the control animals.

One further possibility had to be taken into account; namely, that jaundice might have interfered with the absorption of viosterol at the site of injection. Consequently, tests were made with Drisdol, a water-soluble preparation of "pure crystalline vitamin D_2" in propylene glycol that is more easily and more readily absorbed than oil when it is injected into muscular tissue. These results, however, were identical almost unit per unit with those obtained when viosterol in oil was used (Table I).

The only interpretation remaining, therefore, is to conclude that the impaired liver function was responsible for the decreased antirachitic potency of vitamin D, both in the rachitic rats in which cirrhosis of the liver had been induced by ligation of the common bile duct and in those in which degenerative changes of the liver had been caused by administration of carbon tetrachloride. This conclusion substantiates Gerstenberger's[1] hypothesis.