ficiency in the rat. In general, the changes observed in this study of the rat were similar but much more severe than those reported for the mouse by Levy and Silberberg.[2]

Reasons for the failure to duplicate the Warkany findings (*i.e.* multiple skeletal abnormalities) are not obvious. The experimental conditions and the purified high-carbohydrate diet were similar but not identical with those used by Warkany. The principal dietary difference was the use of an alcohol-extracted casein, probably containing slightly more riboflavin[9] than that used by Warkany, at a 24% instead of 18% level in the diet. The use of a high-fat diet to accentuate the deficiency should have overcome this difference in the protein components of the diets. At the present time 40 litters comprising 350 riboflavin-deficient young have been carefully examined at birth. While clearing with microscopic examination might have revealed some skeletal defects, abnormalities such as shortening of the mandible with protrusion of the tongue and syndactylism of the extremities which occurred with high frequency in Warkany's abnormal young could have been easily recognized by external inspection. Further studies on this problem are in progress.

Summary. The tibias of 6 male rats of the Long-Evans strain born from riboflavin deficient mothers and maintained on riboflavin deficient diets for 3 to 9 months were studied histologically and compared with an equal number of controls reared upon the same diet but with riboflavin added.

In the deficient rats the growth of the tibia was retarded and endochondral ossification was gravely impaired. Marked reduction in chondrogenesis and in capillary erosion and ossification were characteristic for all degrees of the deficiency. The formation of a thin calcified plate on the diaphyseal side of the epiphyseal cartilage is a finding in consonance with the early arrest of growth. Hematopoietic tissue was replaced by fat in all animals after 144 days on the deficient diet.

9 Cannon, M. D., Boutwell, R. K., and Elvehjem, C. A., *Science*, 1945, **102**, 529.

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A New Method for the Production of Non-Specific Capsular Swelling of the Pneumococcus.

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Neufeld,[1] and Neufeld and Etinger-Tulczynska[2] first described the phenomenon of capsular swelling of the pneumococcus when specific antiserum was added to the organism. This capsular swelling, known as the "quellung" reaction, is dependent upon the reaction of capsular polysaccharide with its specific antibody. It has been shown that an excess of specific polysaccharide,[3] heat,[4] papain digestion,[5] and repeated washing with water[6] can produce a reversal of the specific "quellung" reaction.

Non-specific capsular swelling of pneumo-

1 Neufeld, F., *Z. f. Hyg. u. Infectionskrankh.*, 1902, **40**, 54.

2 Neufeld, F., and Etinger-Tulczanska, R., *Ibid.*, 1931, **112**, 492.

3 Nungester, W. J., and Kempf, A. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 705.

4 Etinger-Tulczynska, R., *Z. f. Hyg. u. Infectionskrankh.*, 1933, **114**, 769.

5 Kalmanson, G. M., and Bronfenbrenner, J., *Science*, 1942, **96**, 21.

6 Kempf, A. H., and Nungester, W. J., *J. Inf. Dis.*, 1942, **71**, 50.

TABLE I.
The Effect of Various Proteins and Non-Protein Substances on Capsular Swelling of the Type 27 Pneumococcus.

Substance tested	pH 4.0	pH 5.0	pH 5.5	pH 6.0
Crystalline urease	4+*	0	0	0
Diaphorase	2+	0	0	0
3-Phosphoglyceraldehyde dehydrogenase	0	3+	4+	2+
Purified horse serum albumin	3+	0	0	0
" " hemoglobin	4+	0	0	0
Crude thrombin	4+	0	0	0
Zinc insulin	2+	0	0	0
Cytochrome C	0	0	0	0
" " (dialyzed)	0	0	0	0
Egg albumin	4+	0	0	0
Boiled horse serum albumin	3+	0	0	0
" " hemoglobin	4+	0	0	0
Commercial gelatin	1+	0	0	0
Wheat gluten	2+	0	0	0
Inositol	0	0	0	0
Inulin	0	0	0	0
Lactic acid, M/100	0	0	0	0

* Intensity of capsular swelling is indicated by the conventional notation from 0 to 4+ (maximum).

cocci was first reported by Lofstrom,[7] who used human sera obtained from patients in the acute phase of a febrile, usually bacterial, illness. When such sera were mixed with several different pneumococcal types, capsular swelling occurred. Type 27 pneumococcus was found to react most effectively. Similar acute-phase serum had been shown to produce a precipitate with "C" carbohydrate prepared from a rough strain of pneumococcus.[8-13] Both capsular swelling and the precipitin reaction are reversed by the addition of sodium citrate to remove ionized calcium.[12-14] Reciprocal-absorption experiments revealed that the same reactive protein produced a precipitate with "C" carbohydrate as produced pneumococcal capsular swelling.[13]

Recent work in this laboratory on the "non-specific capsular swelling" properties of human sera for Type 27 pneumococcus has revealed another mechanism for the production of a "quellung" reaction. As far as the author can discover, this mechanism has not been previously described. In the course of experiments to determine the effect of pH on the "non-specific capsular swelling" phenomenon of Lofstrom, acute-phase serum was treated with 0.01 M lactic acid. At pH 5.0-5.5 a protein fraction was precipitated that was partially soluble in an 0.01 M lactic acid solution adjusted to pH 4.0. When formalin-killed Type 27 pneumococcus was added to this soluble fraction at pH 4.0, capsular swelling was observed. All of the human sera tested, normal or abnormal, displayed this reaction. Studies on beef, swine, and lamb serum revealed a similar reaction.

Purified preparations of several enzymes, horse-serum albumin, and horse hemoglobin* were found to react effectively to produce capsular swelling at pH 4.0 (Table I). Test of serial dilutions of the albumin and hemoglobin solutions revealed that the minimal amount of the proteins required to produce "quellung" of the pneumococci was 120 and

7 Lofstrom, G., *Acta Med. Scand.*, 1943, Suppl. **141**, 1.

8 Tillet, W. S., and Francis, T., Jr., *J. Exp. Med.*, 1930, **52**, 561.

9 Tillet, W. S., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1930, **52**, 895.

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11 Abernethy, T. J., and Avery, O. T., *J. Exp. Med.*, 1941, **73**, 173.

12 MacLeod, C. M., and Avery, O. T., *J. Exp. Med.*, 1941, **73**, 183.

13 Lofstrom, G., *Br. J. Exp. Path.*, 1944, **25**, 21.

14 Carlens, E., *Acta Otolaryng.*, 1941, **29**, 316.

* Enzymes and purified albumin and hemoglobin were obtained through the kindness of Dr. Alexander Dounce, Department of Biochemistry, University of Rochester School of Medicine and Dentistry.

20 μg per ml, respectively, when enough organisms were added to give one or 2 pneumococci per oil-immersion field. Non-protein substances and cytochrome C (Table I) were non-reactive. In distinction to the other materials tested, 3-phospho-glyceraldehyde-dehydrogenase was optimally reactive at pH 5.5.

Inhibition of the "quellung" reaction could be brought about by the addition of readily ionized salts to the protein-pneumococcus mixture adjusted to pH 4.0. As low a concentration as 0.02 M sodium chloride and 0.07 M potassium iodide were effective in inhibiting capsular swelling. On the other hand, the reaction was not inhibited in 0.55 M glucose.

When purified horse hemoglobin was used to produce capsular swelling, it was noted that condensation of the protein occurred around the enlarged capsule. This gave the appearance of a distinct rim of golden pigment dotting the periphery of the capsule. As the reaction proceeded, each pneumococcus was completely surrounded by hemoglobin. Eosin azo-dog serum, in a similar manner, produced capsular swelling. The protein appeared to penetrate the capsule to give it a diffusely stained, deep-pink color. When specific antiserum was added to maintain capsular swelling and the pH was brought back to 7.0, the pink-staining protein was no longer present in the capsule. Pneumococci were treated with eosin azo-protein at pH 4.0, centrifuged free of the solution, brought to pH 7.0 and then placed in a colorless protein solution at pH 4.0. These organisms then contained no microscopically visible eosin azo-protein in the swollen capsular substance. The reaction would appear to demonstrate a reversible combination of protein with the capsular polysaccharide, dependent on conditions of pH.

This new method of producing non-specific capsular swelling is not peculiar to Type 27 pneumococcus. Formalin-treated pneumococci, Types 1, 2, 3, 5, 7, 8, 19, and 28 were tested and all reacted with equal facility to several proteins. Similar capsular swelling did not occur, however, with *Klebsiella pneumoniae*, a mucoid *Escherichia coli*, or a mucoid *Streptococcus hemolyticus*, although acid agglutination was observed under these conditions.

An attempt was made to determine the reactivity of purified polysaccharide substances with different non-specific proteins at pH 4.0. Both Type 28 and Type 3 polysaccharide substances were found to be reactive with proteins at pH 4.0 to produce a dense flocculent precipitate. This combination was reversible in that the precipitate washed in 0.01 M lactic acid solution adjusted to pH 4.0 and then brought to pH 7.0 was found to be clear and to react by precipitation with Type 3 specific antiserum. A pH of 4.0 was well below the iso-electric range of the proteins tested, and the pH of each protein-carbohydrate reaction mixture was redetermined to insure that flocculation was not due to iso-electric precipitation of the protein. The addition of sodium chloride did not inhibit precipitation, in distinction to the observed effect on the "quellung" reaction.

Discussion. The mechanism of the "quellung" reaction is not clear. Johnson and Dennison[15] felt that the increase in volume of the pneumococcus resulting from "quellung" was greater than the aggregate volume of the antibody molecules adherent to the capsule. They suggested that capsular hydration took place as a result of antigen-antibody combination. Mudd, *et al.*,[16] on the basis of electron microscopy, felt that the pneumococcal capsule consists of a gel of low density that reacts with homologous immune serum to produce increased thickness and density of capsular gel. Kempf and Nungester[6] noted that a 2 molar solution of sodium chloride did not prevent or reverse the "quellung" reaction to specific serum. The enlarged capsules disappeared on washing several times in water, only to reappear with the addition of physiological saline solution.

Summary. The experiments outlined indicate that pneumococcal polysaccharides combine with various proteins at pH 4.0 to form

15 Johnson, F. H., and Dennison, W. L., *J. Immunol.*, 1944, **48**, 317.

16 Mudd, S., Heinmets, F., and Anderson, T. F., *J. Exp. Med.*, 1943, **78**, 327.

insoluble aggregates. This reaction is one of loose combination, since it can be reversed by readjusting the pH to 7.0. Non-specific capsular swelling of pneumococci can be produced also under these conditions, but, in addition, the protein-pneumococcus mixture at pH 4.0 must be relatively salt-free. It is felt that an altered state of the capsular gel is produced by the interaction of protein and carbohydrate. In the absence of ionized salts, the polysaccharide-protein gel becomes increasingly hydrophilic and therefore capable of enlarging to produce the "quellung" reaction. Both specific and the described acid-protein quellung can be produced in the absence of calcium in distinction to Lofstrom's non-specific "quellung" phenomenon.

Grateful acknowledgment is made to Henry W. Scherp, Ph.D., Department of Bacteriology, University of Rochester School of Medicine and Dentistry, for supplying the polysaccharide preparations and eosin azo-protein, and for many helpful criticisms and suggestions.

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Effect of Alloxan Diabetes on Reproduction in the Rat.*

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The administration of alloxan to experimental animals will produce a condition analogous to diabetes mellitus in man.[1,2,3] The principal effect of the drug is a selective necrosis of the islets of Langerhans in the pancreas, although there is slight damage to other tissues.[4,5] The experimental diabetes may be transitory in character and the animal recover, or it may be permanent, becoming progressively more severe until the animal succumbs. The dose of the alloxan administered is the most important factor in the ultimate course of the diabetic state.

Friedgood and Miller[6] injected alloxan 4 days before parturition and obtained normal litters. This observation suggests that terminal diabetes is not sufficient to interfere with the development of fetuses. The high incidence of pregnancy complications in human diabetics prompted us to restudy the problem and the following experiments were designed to provide additional pertinent information.

Methods. The objectives of these experiments were three-fold: (1) to determine the effect of alloxan diabetes on cyclical activity and fertility in the rat, (2) to study reproduction in rats made diabetic early in pregnancy and (3) to evaluate the course of pregnancy and labor in the rat with diabetes controlled adequately by insulin.

Young mature female rats, weighing 175 to 250 g, of the inbred Sprague-Dawley stock raised and maintained in our laboratory were used throughout these studies. They were given free access to a balanced diet which was supplemented by milk as well as wheat germ oil once a week. Sex cycles were followed routinely employing the standard vaginal

* This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

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2 Dunn, J. S., and McLetchie, N. G. B., *Lancet*, 1943, **2**, 384.

3 Goldner, M. G., and Gomori, G., *Endocrinology*, 1943, **33**, 297.

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5 Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B., *Lancet*, 1943, **1**, 484.

6 Friedgood, C. E., and Miller, A. A., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 61.