can be further purified by reprecipitation 2 or 3 times. If one is not interested in preparing a soluble preparation, the yield can be greatly increased by allowing a longer time for precipitation with the acetic acid.

This procedure has several advantages over that of Oswald. The time is greatly reduced since no filtering or washing is necessary. The hemoglobin is not precipitated as it is with ammonium sulfate. The dilution 10 times and the slow formation of the precipitate allow many impurities to remain in solution. The cost is negligible even on a large scale. The isoelectric point is being used to purify thyroglobulin.

6035
Colony Forms of B. Paratyphosus B as Related to Variations in Gas Production.

W. J. NUNGESTER AND RUTH WESTLUND JUNG.

From the Department of Bacteriology, Northwestern University Medical School.

It is the purpose of this paper to describe variations in colony form and gas production that occurred in a stock culture of *B. paratyphosus B.* and particularly to emphasize a lack of correlation between these 2 properties.

Variations in properties of organisms of the paratyphoid group have been the subject of numerous studies. Jordan[1] has investigated colony form and its relation to virulence. Variation in the immunological properties of this group has been studied by the English workers.[2] Biochemical variations, particularly the failure of certain strains to form gas from carbohydrates was noted by Oette.[3] Herrmann[4] more recently isolated 16 strains which failed to form gas from dextrose and other fermentable carbohydrates.

During the course of experimental work irregular results in gas production from dextrose by a stock strain of *B. paratyphosus B.* led us to plate out the culture. The colonies were predominantly of the R type with a scattering of the S variety. A preliminary study of cultures of the S and R varieties with respect to their ability to form gas, resulted in subdividing both the R and S forms into

[1] Jordan, *J. Am. Med. Assn.*, 1926, **86**, 177.

[2] White, P. Bruce, A System of Bacteriology, London, 1929, V. 4.

[3] Oette, *Centralbl. f. Bakteriol.*, I. O., 1913, **68**, 1.

[4] Herrmann, *Centralbl. f. Bakteriol.*, I. O., 1929, **113**, 108.

strains which produced gas from dextrose and strains which failed to show this physiological activity.

As a result we had on hand 2 rough colony types Rg+ and Rg— which respectively did and did not form gas from dextrose. Also the cultures Sg+ and Sg— varied from each other in an analogous manner. These variants have been carried on solid medium for 4 months with no apparent change in investigated properties.

The biochemical properties: All forms produced acid from dextrose, maltose, mannite, galactose, xylose, and levulose and failed to produce acid from lactose, sucrose, raffinose, dextrin, and inulin. The fermentation tests were made both on carbohydrate Andrade agar and in carbohydrate broth. The variants Rg— and Sg— which failed to form gas from dextrose also failed to form gas from the other carbohydrates which they utilized with acid production. The variants Rg+ and Sg+ formed gas from all utilizable carbohydrates. No difference was noted in the rate of acid production of the variants in dextrose. All forms darkened lead acetate medium equally. Gelatin was not liquified after 16 days. No form produced indol. In indicator milk all forms produced an initial acidity without coagulation. The S variants caused a shift in the milk reaction to the alkaline range after 8 days incubation. An acid reaction was still present in the milk inoculated with the R form after 16 days.

All forms were agglutinated in a dilution of 1-1600 by a commercial anti-para B. serum. No significant agglutination was noted with anti typhosus or anti para-typhosus A. sera.

Morphologically the variants were characterized as Gram negative, non-spore-forming, motile rods.

To study the change in biochemical and colonial properties of the various forms, they were inoculated into mediums as indicated in the table and plates were streaked from these cultures at designated intervals. Colony forms were noted and 10 single colonies were subcultured to dextrose broth to determine the percent of gas-forming organisms present in the aging culture of the variant.

But limited changes in colony form of the cultures were noted during these studies. Intermediate forms, to the extent of 5-10% and an occasional S colony, occurred on plates inoculated from the aging R cultures. Intermediate and rough colony types appeared to a limited degree on plates streaked from the S. cultures. On further examination of the colonies for gas-forming cultures, as indicated above, less than 1% of the 500 colonies studied, arising from subcultures of the aging Sg+ and Rg+ forms failed to form

gas. These gas negative cultures arose from colonies essentially identical with those of the inoculated gas-forming type. The colonies occurring on these plates which were atypical of the inoculated form produced gas in all cases tested.

The non-gas-forming variants after 8 or 10 days incubation be-

TABLE I.

Percent of Colonies from Aging Cultures of Variants which Produced Gas when Inoculated into Dextrose Broth.

Date Inoculated	10-21-31				10-25-31				11-24-31				1-12-32			
Medium	100 cc. Plain Broth				10 cc. 20% Serum Broth				100 cc. Plain Broth				100 cc. Plain Broth			
Variant	Sg+	Sg—	Rg+	Rg—	Sg+	Sg—	Rg+	Rg—	Sg+	Sg—	Rg+	Rg—	Sg+	Sg—	Rg+	Rg—
Age of Culture day																
1	100	0	100	0	100	10?	90	0					100	0	100	0
2	100	0	100	0					90	0	100	0	100	0	100	0
4													100	0	100	0
6					90	0	100	0	100	0	100	0				
10	100	50	90	0	100	50	100	0					100	0	100	0
15													100	90	100	40
18	100	100	100	30	100	90	100	10	100	90	100	70	100	90	100	100
28	100	100	100	60	100	30	100	20	100	100	100	90				
52	100	100	100	100												

gan to yield an increasing number of colonies which, when examined, indicated the presence of gas-producing organisms. There was a lack of correlation between changes in colony form and gas production. Colonies atypical of the inoculated variant might or might not yield a gas-producing culture. The same was true for the colonies of the type inoculated.

Conclusion. The evidence presented seems to be in accord with the concept that bacterial properties may vary independently of each other.

6036

A Reducing Substance in the Urine of Cats Under Nembutal Anesthesia.

R. W. BARRIS AND H. W. MAGOUN. (Introduced by S. W. Ranson.)

From the Institute of Neurology, Northwestern University Medical School.

A series of 15 cats were given intraperitoneal injections of nembutal (sodium iso-amytal, penta-barbital), the average dose being 60 mg. per kilo. Urine samples were collected at intervals after the injection and later analyzed for reducing substances by Sumner's method.[1] In certain instances the results thus obtained were checked by the use of Benedict's quantitative reagent. Simultaneously blood samples were taken and later analyzed for "blood sugar" by the Randle-Grigg modification of the Folin-Wu Micro Method.[2] These observations were made in the course of experiments to determine the effect of stimulation of the *tuber cinereum* upon carbohydrate metabolism. Rather extensive surgical procedures under deep nembutal anesthesia were required in the experiments. The bladder was exposed and emptied and the urethra cannulated from one to 2 hours after the injection of nembutal. The first sample of urine was taken about an hour after the urethra was cannulated and from 2 to 3 hours after the administration of nembutal. This sample always showed the presence of an abnormal quantity of reducing substance, usually the maximum obtained during the experiment, the concentration decreasing progressively in subsequent samples. This maximum reduction where the lowest maximum was obtained, was equivalent to that produced by 240 mg. % of glucose. In the experiment where the largest maximum was found, the reducing sub-

[1] Sumner, *J. Biol. Chem.*, 1925, **65**, 383.

[2] Randles, F. S., and Grigg, W. K., *J. Am. Med. Assn.*, 1924, **82**, 684.