method. Interestingly enough, when there is a large amount of glycogen in the cells near the periphery of the liver lobule, it appears to be most concentrated in the cytoplasm adjacent to the sinusoids (Fig. 1 and 4).

The appraisal of the amount of glycogen in histological preparations is hampered by the unequal zonation of glycogen in the liver lobule. It was also found that lobules of the same liver lobe may possess different quantities of glycogen. This is true even when the sections are entirely uniform in thickness. Moreover, the 2 lobes occasionally present significantly different levels of glycogen (*e.g.*, No. 6b, 12c, and 24c). The possibility of such differences in distribution needs to be taken into consideration by both histologists and biochemists in drawing conclusions from small samples of liver.

The analytical data (Column 7) and the average corrected photometer readings (Column 5) of the livers of the individual rats are recorded in Table I by groups, according to the lapse of time after the last feeding. The average ratio of glycogen found/photometer reading = 0.164. Multiplying the individual photometer readings by this factor gives a calculated value of the glycogen in the histological sections of each rat liver (Column 6). The standard deviation of the differences between the found and calculated glycogen values is ± 0.52.

The data for chemically determined glycogen have also been plotted against the corrected photometer readings in Fig. 5. The black dots designate the average of the optical densities of the sections of a liver and the corresponding chemical glycogen determinations. The horizontal lines through the dots indicate the differences between photometer readings for the right and left lobes. It is seen that up to a glycogen concentration of about 5% a linear correlation exists between the 2 methods of determination.

It may be further pointed out that though no significant difference occurred in the liver glycogen between the 6- and 12-hour postfeeding periods, a marked progressive decrease occurred between the 12- to 18- and the 18- to 24-hour periods.

Summary. Glycogen of rat liver was well preserved throughout histological blocks and was not displaced in the cells when pieces of liver were fixed in ice-cold picro-alcohol-formalin. After fixation, liver sections were stained by the Bauer-Feulgen method and the optical density of each section was measured on a Photovolt electronic photometer. Chemical determinations were made of the glycogen contained in other pieces of the same livers. The livers of rats undergoing progressive starvation from 6 to 48 hours showed good correlation between the chemically and histologically determined glycogen concentrations.

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A Virus Causing Abnormal Milk in Cattle.

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Dairy cows near Princeton, N. J., have shown an unusually high incidence of bloody thickened milk during the spring, summer and fall of 1946. The majority of cases occurred in the latter part of May and in June and animals in all stages of lactation were affected. Many specimens of abnormal milk contained flakes composed of polynuclear and mononuclear cells. Affected animals frequently showed temperatures exceeding 40°C but otherwise appeared normal and continued to eat as usual. In most cases all visible signs disappeared after 2 to 4 days. These facts suggested an infectious nature and in-

vestigations were made in an attempt to procure the etiological agent.

Guinea pigs weighing 200 to 300 g and kept under constant conditions of temperature (70°F) and humidity (60%) were inoculated intraperitoneally with either 1 or 5 cc of milk freshly drawn from naturally infected cows. From each of 3 milk samples furnished by different cows an agent was established in guinea pigs that caused temperature elevations exceeding 40°C beginning 4 to 7 days after inoculation and lasting for 3 days. Other than fever no definite signs of illness were noted, and all animals survived unless killed for further study. Animals killed during the febrile period showed hemorrhages in the lungs, focal necrosis of the liver and slightly enlarged mesenteric lymph nodes. These findings remained constant through 20 serial passages in guinea pigs maintained by blood transfers. Following recovery animals given a second inoculation with the same strain showed no response and in cross inoculations between the 3 strains it was found that each immunized against the others. Therefore presence of the agent was determined by a typical thermal response in guinea pigs, followed by subsequent immunity to test inoculation with a reference strain.

The agent was transferred from guinea pigs to rabbits by intravenous and to mice by intraperitoneal inoculation. Rabbits responded with a thermal reaction similar to that in guinea pigs. Mice showed no evidence of infection but tests showed that the agent was present after 3 serial passages. The agent was maintained for 1 but not 2 serial passages when inoculated into the yolk sac of 5-day embryonated hen's eggs. When inoculated on the chorioallantoic membrane of eggs that had been incubated for 10 days, tests showed that the agent was present after 10 serial transfers. No pathological alterations of the membrane were noted.

A lactating cow inoculated subcutaneously with 1 cc of blood from the first guinea pig transfer showed fever 7 days later, and a second lactating cow inoculated in the same way with blood from the 6th guinea pig passage showed fever on the 9th day. While both animals showed fever and alterations in the milk similar to those seen in natural cases, the milk did not contain red blood cells. The agent was demonstrated in the blood of both animals and in the milk of the first cow. Similar inoculations of the agent into 2 young calves produced a febrile reaction and tests showed that the blood contained the agent. Sera of animals recovered from either experimental or natural disease neutralized 100 to 1000 infectious doses for guinea pigs, whereas sera from normal animals and those in the acute phase of illness failed to neutralize 1 to 10 infectious doses.

Films from yolk sac membranes in the first passage and infected chorioallantoic membranes stained with methylene blue and by Macchiavello's method showed no organisms. Transfers of blood from infected guinea pigs and of infected chorioallantoic membranes showed no growth on blood agar slants and sealed cooked meat medium. This failure to demonstrate the agent microscopically or culturally and its specificity for the chorioallantoic membrane indicated that the agent was a virus.

From the work reported herein, it appears that a virus has been secured in guinea pigs from abnormal milk of diseased dairy cattle. When blood from infected guinea pigs was inoculated subcutaneously into lactating cows, a disease was produced resembling that seen in animals from which the virus came originally. Neutralizing antibodies developed in experimental cows and calves, and sera from cows that had recovered from the natural disease also neutralized the agent. Various workers have postulated that a virus may initiate some types of bacterial mastitis.[1] Further work must be done to determine whether this virus can act in such a manner.

[1] Little, R. B., and Plastridge, W. N., *Bovine Mastitis, A Symposium*, New York, McGraw-Hill Book Co., Inc., in press.