An interval of 30 min between bleeding and centrifugation was found to be sufficient for obtaining a high yield of serum, provided that clotting set in within 5 min. Blood samples with long clotting times (10–60 min), however, frequently gave very low yields of serum when centrifuged 30 min after clotting had set in. Three, instead of two, layers were formed on centrifugation—blood cells, plasma clot, and serum. It was possible to shorten the clotting time to 2–3 min and prevent the plasma clot formation by the addition of one drop of species specific thromboplastin reagent[1] per 8 ml. of blood. Plasma clots could also be prevented in samples with long clotting time if the clot was allowed to stand for 16 h before centrifugation. The addition of thromboplastin to blood samples with short clotting times (1–5 min) had no effect on the serum yield.

The addition of 0·1 ml of M/5 calcium chloride/8 ml. blood had no effect on either the clotting time or the serum yield, even when samples with 60 min clotting times were used. Blood samples of long clotting time were obtained by catheterization of the brachial vein[2]. Budtz-Olsen reported that, between 4° and 39° C, the higher the temperature the greater the degree of human clot retraction[3]. Thus serum yields after centrifuging (2,400*g*) chicken blood at 20° C and at 40° C were compared. No appreciable difference was detected. Neither the size (12 ml. or 50 ml. capacity) nor the type of the collecting vessel (glass, cellulose nitrate or polypropylene) made any appreciable difference on the serum yield when high centrifugal force was used.

The foregoing procedure of obtaining serum was found applicable to rabbit, guinea pig and hamster, as well as to chicken blood.

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PATHOLOGY

Plaque-size Mutants obtained from the Ribonucleic Acid Phage *fr* after Treatment with Nitrous Acid

PHAGE *fr* (formerly *ft* 5 (ref. 1)) in its characteristics[2] is related to *f*-phages described by Loeb and Zinder[3]. For the initiation of genetical studies, attempts were made to obtain mutants from phage-suspensions treated with nitrous acid. Conditions of treatment were essentially those described by Schuster and Schramm[4]. Single-hit kinetics of inactivation was established over a range of 5 logarithmic cycles. Standard phage techniques[5] were used. Since the rate of inactivation is exponential in relation to the time of incubation in nitrous acid, the inactivation is characterized by the 'reduced time' $\tau = e^{-1}$. When suspensions of *fr* were inactivated at 37° C and *p*H 4·28 by 0·5 M sodium nitrite, τ equalled 3·5 min. A comparison with the rates of inactivation by nitrous acid as reported for other viruses is shown in Table 1. It demonstrates that the τ of inactivation of phage *fr* is more closely of the order of the τ of tobacco mosaic virus (TMV) than that of DNA-phages.

Table 1. COMPARISON OF THE INACTIVATION-RATE BY NITROUS ACID OF PHAGE *fr* AND OTHER VIRUSES

Virus material	Temperature (° C)	*p*H	$NaNO_2$ (molarity)	τ	Reference
TMV	22	4·2	1·0	23	9
TMV—RNS	22	4·5	1·0	8·5	9
fr	20	4·28	0·5	12	—
T_2	20	4·3	0·05	13·0	6
T_2	20	4·55	0·5	2·06	7
$\varphi X174$	25	4·0	0·05	10·5	8

Using the pre-adsorption technique platings of phage *fr* always showed a few smaller plaques (1 mm diam.) in the presence of predominantly larger ones (3–6 mm diam.). If such small plaques were isolated and replated, it could be shown that this characteristic of their plaque morphology was not stable. However, with increasing time of exposure to the action of nitrous acid, in the platings the proportion of small plaques increased. A number of such small plaques were isolated. Replating these isolates revealed that the fraction of stable small-plaque clones was becoming larger with increasing time of exposure to nitrous acid: after 20 min incubation 25 per cent, after 60 min incubation 80 per cent of the small plaques isolated gave stable small-plaque clones. A number of such stable small-plaque mutants were re-isolated for further characterization and lysates were prepared. The isolates proved to be closely related to wild-type in their rate of inactivation by nitrous acid, their burst size, and their inactivation by *fr* antiserum. Furthermore, if RNase is present in the adsorption mixture, plaque yields of these mutants are reduced to the same extent as those of wild-type plaques.

Phage *fr* was obtained from Dr. Hoffmann-Berling, Max-Planck-Institut für medizinische Forschung, Heidelberg.

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Muscle Glycogen in Juvenile Diabetes before and during Treatment with Insulin

IT is well known that insulin stimulates the synthesis of glycogen in isolated muscle tissue from different animals. Insulin is considered to increase the permeability of the cell membrane to glucose. Direct action on the enzymes responsible for the synthesis of glycogen has also been observed. Low muscle glycogen has been found in animals with experimental diabetes. There are, however, few reports on the muscle glycogen content in normal and diabetic man. The glycogen content in muscle biopsy specimens from normal subjects was investigated by Hildes *et al.*[1–3] and by Nichols[4]. Hildes *et al.*[2,3] also determined the glycogen content in muscle tissue from patients with diabetes. No significant decrease was found in the muscle glycogen compared with the normal subjects. Their patients were adults who, in most cases, had been treated with insulin up to a few days before the examination.

This communication reports results from a material consisting of six patients with uncompensated juvenile diabetes. Muscle glycogen was determined before and during insulin treatment.

In four of the cases the diabetes was newly discovered and no insulin had been given before the first examination. Two patients had received insulin treatment for six months; but due to exacerbation of the disease, their insulin doses were insufficient for some weeks before the first examination. In one patient (No. 3) the diabetes was detected by routine examination for glucose of the urine. All the other patients had classical symptoms of diabetes. Metabolic acidosis was present in four cases.

Muscle biopsies were performed immediately before the administration of insulin and after 9–16 days' treatment