"Failure to respond to 100 STD" would indicate the order of average resistance acquired under the conditions of the procedure of immunization just described.

Parallel with some of these series, animals were injected with the same toxin treated with 0.3% formol until well over 90% of its toxicity as measured in the rabbit skin had disappeared. Some immunizing effect was observed (Table I), but markedly less than with the original toxin. It is irrelevant for the moment whether this remaining effect was due to the traces of unmodified toxin still present. The purpose of reporting these experiments here is to show that quantitative differences in immunizing effect can be detected by the intradermal test.

We recommend to test the acquired resistance one week after completion of immunization. At this time, dermal resistance is at its high point. Animals tested 2 weeks after completion of immunization already showed signs of lessening of resistance.

As a preliminary standard for comparative experiments the immunizing effect of scarlet fever toxin in amounts as used for human immunization might form a useful basis. It is obvious from the tables that 6 to 10 animals should be employed in each series to guard against deception by individual variation.

Dermal resistance to toxin can also be obtained by passive immunization. Two groups of rabbits were injected with commercial scarlatinal antitoxin (Globulin Modified, Lederle), in a dosage of 1 U.S.P.H.S. unit per g body weight. At the same time, rabbits were injected with the same amount (measured as serum protein) of commercial diphtheric antitoxin (Globulin Modified). Twenty-four hr later, all animals were tested with graded amounts of toxin in the skin. Results can be seen from Table II. It will be seen that a dermal resistance similar in degree to that obtained with active immunization with scarlet fever toxin was obtained after passive immunization with scarlatinal antitoxin, whereas injection with diphtheric antitoxin would not change Dick sensitivity.

Summary. Dermal resistance to graded amounts of Dick toxin can be used as a method for evaluation of agents for scarlet fever immunization.

13960

Simplified Technic for Inoculating into Amniotic Sac of Chick Embryos.*

R. M. TAYLOR AND R. J. CHIALVO. (Introduced by J. H. Bauer.)

From the Virus Department of the Instituto Bacteriológico, Buenos Aires.

Burnet[1] has reported the isolation of influenza virus direct from throat washings, by inoculation into the amniotic sac of chick embryos and subsequent passages with a suspension of the ground tracheas removed from the embryos following a suitable period of incubation.

The technic employed by Burnet involves cutting a window in the egg shell over the embryo, puncturing the shell membrane and transferring the air sac to between the shell and the chorioallantoic membranes beneath the opening. The cut portion of the shell, including the attached shell membrane, is then removed exposing the chorioallantois beneath the window in the shell. A small slit is made in the chorioallantois, and by means of a fine forceps the underlying amniotic membrane is grasped and drawn through the opening in the chorioallantois. Inoculation is made into the amniotic sac by puncturing the exposed membrane with a capillary pipette. The membrane is then released and allowed to withdraw through the opening in the chorioallantois. Finally, the window in

* These studies were supported in part by a grant from the International Health Division of The Rockefeller Foundation.

1 Burnet, F. M., *The British J. Exp. Path.*, 1940, **21**, 147.

the shell is closed with a cover glass and the edges sealed with paraffin. Unless a very large opening is made in the shell at the time of the inoculation, it is necessary to enlarge it further, following incubation, for the removal of the embryo.

Experimental. We have found the following technic to be somewhat simpler than the one described by Burnet.

During transillumination of the egg to determine whether the embryo is alive, the border of the air sac is marked upon the shell with a pencil. The shell under the circular pencil mark is then cut through with a dental drill, leaving the shell membrane intact. Alcohol is applied, the exposed shell membrane is cut through with sterile scissors,

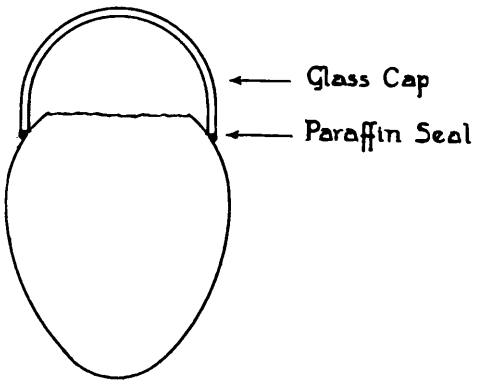

FIG. 1.

and the shell cap over the air sac is removed, thus exposing the diverted layer of the shell membrane which covers the chorioallantois. While dry the exposed membrane is quite opaque, but if a drop or two of 50% alcohol is placed upon it, it becomes sufficiently translucent to make visible the border of the yolk sac and the larger blood vessels in the chorioallantois. Avoiding the yolk sac and the blood vessels, the closed points of a fine curved forceps are thrust through the outer membrane and chorioallantois. The forceps are then slightly opened, and the underlying amniotic sac is grasped and pulled through the chorioallantois and outer membrane. While the tent-like fold of the amniotic membrane is being held by the forceps, inoculation is made into the base of the exposed sac with a tuberculin syringe attached to a fine needle (27 gauge). The fold of the membrane is released and of its own accord slips back into place.

The circular opening is then covered by an inverted glass cup with an inside diameter of 32 mm (Fig. 1). In order to obtain a hermetic seal the brim of the cup is dipped in melted paraffin (melting point 45°) just before placing it upon the egg. The eggs are incubated in an upright position (cup end upward).

When it is desired to examine the embryo following incubation, the cup is removed, the shell membrane and the chorioallantois are cut away, and the embryo is lifted out with a pair of forceps and placed in a Petri dish. No further enlargement of the opening in the shell is required.

If it is desired to examine the allantoic fluid, this is first withdrawn by means of a 10 cc syringe with a 22 gauge needle. The needle is thrust through the shell and chorioallantoic membranes near the side of the shell, and the fluid is aspirated. It is usually found to be clear and free from red blood cells. Following withdrawal of the allantoic fluid, the membranes are cut away with scissors thus exposing the embryo and the amniotic sac. The amniotic fluid may be obtained by either puncturing the sac *in situ*, or after it is removed with the embryo.

Occasionally an egg is encountered (not more than one in 10) in which the yolk sac almost completely covers the under surface of the air sac. In such an event some difficulty may be experienced in seizing the amniotic sac and drawing it to the surface, but this can usually be accomplished.

In our experience the advantages of this method are: (1) it is not necessary to determine in advance the exact location of the embryo. (2) The shell can be cut about the natural air sac with greater facility than over the embryo, as there is no danger of injuring the blood vessels of the chorioallantois. (3) It does not require the transference of the air sac. (4) The allantoic liquid may be easily removed free from blood cells. (5) By means of an inverted glass cup the opening in the shell can be easily and hermetically

sealed. (6) No further enlargement in the opening in the shell is required for removal of the embryo.

Using this technic of inoculating eggs, we have succeeded in isolating influenza virus A from throat washings which had been preserved at a temperature of approximately —76° for nearly 2 years.

13961

Influence of Estradiol Benzoate on Fat Storage.*

HAROLD G. LOEB.† (Introduced by G. O. Burr.)

From the Departments of Physiological Chemistry and Botany, University of Minnesota, Minneapolis.

In a recent study Loeb and Burr[1] showed that female rats store more body fat than males when maintained on a diet rich in saturated fat (hydrogenated coconut oil) but devoid of essential fatty acids. It then became a matter of interest to ascertain whether this finding could be attributed to the effect of estrogen on fat metabolism. Zondek and Marx[2] reported that estrogen not only induced a marked lipemia in fowl but also caused the accumulation of fat in the internal organs, particularly the liver. However, their findings with mammals were negative. Loeb[3] observed a definite, though moderate, lipemia in male rats fed a high fat diet lacking in essential fatty acids when estradiol benzoate was injected over a 4-week period. The present report is concerned with the storage of body fat under similar conditions.

Methods. Albino rats of the Wistar strain were taken at weaning time and placed on a low fat diet for 4 weeks to deplete the fat reserve. During the next 8 weeks, which comprised the experimental period, the animals were maintained on diet 580-B, a ration rich in saturated fat—71% hydrogenated coconut oil. One group received, subcutaneously, 3 large doses of estradiol benzoate‡ (100 μg per dose§), on alternate days, during the last week of the experimental period. Two other groups were given 24 more moderate doses (30 μg and 5 μg, respectively), daily except Sunday, throughout the last 4 weeks of the experimental period.[3] The animals were sacrificed by etherization and the carcasses† analyzed for total fat by digestion with 28% KOH in 50% alcohol for 4 hr followed by petroleum ether extraction of acidified aliquot portions. All analyses were done on duplicate aliquot portions which checked well within 1%.

Table I shows the mean values for total carcass lipid of the groups investigated. It will be seen that female controls (received no estradiol benzoate) had an appreciably higher carcass fat than the males. Rats which received 3 large doses of the hormone during the last week had a lower fat content than their male controls; but this effect was due to diminished food consumption under the influence of large doses of estrogen. Group C showed a higher content of body

* Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration, Official Project No. 265-1-71-236, Sub-project No. 382.

† Rockefeller Foundation Fellow.

1 Loeb, H. G., and Burr, G. O., unpublished results.

2 Zondek, B., and Marx, L., *Arch. Int. Pharmacodyn.*, 1939, **61**, 77.

3 Loeb, H. G., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 340.

4 Fisher, R. A., *Statistical Methods for Research Workers*, 7th Ed., Oliver and Boyd, London, 1938.

‡ The author wishes to thank Dr. R. G. Floody (Roche-Organon, Inc.) who kindly supplied the estradiol benzoate.

§ Each dose in every case was contained in 0.05 cc of olive oil.

† The carcass was prepared for analysis by removing the head, tail, and limbs.