proved to be completely unreliable, both controls and treated animals showing a high percentage of complete regressions. Of a group of 27 mice which received no injections, 19 showed complete disappearance of the implanted tumor after the tumor had reached a size of one cm in largest diameter.

These results do not confirm those of Klyueva and Roskin as given in the fragmentary reports available to us. At no time did we have detailed information regarding their methods of *T. cruzi* cultivation and lysate preparation. It is possible that the discrepancy may be due to differences in the ultimate nature of the lysate.

Summary. 1. No inhibition of tumors was noted in Bagg-albino strain mice with spontaneous mammary carcinoma over one cm in size when treated with whole culture lysate of *T. cruzi;* on the other hand in the same mouse strain with tumors under one cm in size a degree of inhibition was noted which warrants closer study.

2. The inhibition noted could not be attributed to malnutrition effects or concurrent bacterial infections.

3. No inhibition was noted in tumor-bearing "A" strain mice (spontaneous mammary carcinoma; transplanted carcinoma No. 119) when treated with *T. cruzi* whole culture lysates.

4. Survival time of treated tumor-bearing mice was less than that of control treated animals.

5. A simple method is given for a reproducible preparation of *T. cruzi* lysates.

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Propagation of Rabbit Fibroma Virus in the Embryonated Egg.*

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In view of the theoretical interest attached to the relationship of viruses to neoplasms,[1] it seems worth while to report the adaptation to growth in the embryonated egg of a virus which, if it does not cause a true tumor, is at least the etiologic agent of a closely related tumor-like condition. Adaptation of such a virus to a different and relatively simple experimental host such as the developing chick embryo may provide a means of studying the virus-tumor relationship. In fact the earliest use of the chick embryo in the study of viruses was for just this purpose, when Murphy and Rous obtained growth of a fowl sarcoma on inoculating into the chick embryo either minced tumor tissue or a Berkefeld filtrate of such tissue.[2] The virus studied here is that of rabbit fibroma isolated by Shope[3] from a growth on the foot of a wild rabbit. Passed since then in series through domestic rabbits, one strain (the so-called OA strain) has maintained its ability to produce tumors on subcutaneous or intratesticular inoculation. From this original strain there arose a variant (the so-called IA strain described by Andrews[4]) which had lost the ability to produce tumors and caused only

* Part of work reported here was carried out while the author was a National Research Council Fellow in the Medical Sciences.

1 Rous, P., *Bull. New York Acad. Med.*, 1947, **23**, 65.

2 Murphy, J. B., and Rous, P., *J. Exp. Med.*, 1912, **15**, 119.

3 Shope, R. E., *J. Exp. Med.*, 1932, **56**, 793.

4 Andrewes, C. H., *J. Exp. Med.*, 1936, **63**, 157.

inflammation.

After many unsuccessful attempts at growing strains of the fibroma virus in tissue culture, Faulkner and Andrewes[5] finally succeeded in adapting the IA strain to growth in cultures of rabbit testis.

Paschen,[6] was unable to maintain the IA strain on the chorioallantoic membrane for more than one passage, although the OA strain was still present after 5 passages. However methods and findings are not given in any detail.

Experimental. Methods. The *strain of virus* employed in most of our experiments was the OA, or tumor-producing strain, obtained from Dr. R. E. Shope as glycerinated rabbit testicle. The IA strain was used similarly in the form of glycerinated rabbit testicle. *Titrations* were carried out by grinding a weighed piece of tissue with sand in a mortar and adding sufficient cold veal infusion broth or saline to make a 10% suspension. Further tenfold dilutions were then made in cold broth, and inoculated subcutaneously in a rabbit in 1.0 cc amounts, no more than 9 tests being carried out in any one animal. The rabbits were observed for appearance of subcutaneous tumors at the site of inoculation. *Neutralization* tests were carried out according to Shope's[7] method, *i.e.* a mixture of 1 cc of serum and 1 cc of infection suspension was incubated at 37°C for 1 hour, stored for 2 hours in the refrigerator, then injected in 1 cc amounts into the subcutaneous tissue of a rabbit, together with appropriate controls in the same rabbit.

Results. Adaptation of the Virus to Eggs. The stored glycerinated testicle virus was well washed in cold saline, then ground with sand and saline to make a heavy suspension, which was used to infect a fresh rabbit, 0.5 cc being inoculated into each testicle. Seven days later, when scrotal and testicular edema were pronounced, the animal was killed, small pieces of testicle were ground with sand and sufficient cold saline to make a 1% suspension by weight. After this suspension had been allowed to settle for about an hour in the refrigerator, 0.1 cc of the suspension was dropped onto the chorioallantoic membrane of 4 12-day-old eggs and was also cultured on a blood agar slant. Three days later, after incubation at 35°C, the membranes were harvested, ground lightly in a pyrex glass grinder, together with 4 cc of saline per membrane. After standing for half an hour the supernatant of this suspension was used as inoculum for a new set of eggs. Passages were made in this fashion at regular 3-day intervals. The membrane suspension from the 7th egg passage produced typical fibroma in a rabbit. The egg passage virus having unfortunately become contaminated at the 8th passage, the testicle from the rabbit injected with 7th passage egg membrane suspension was used as a source of virus. The virus was then maintained in eggs for 18 consecutive passages without mishap. In this second series membranes of the 1st, 5th, 10th, 14th, 15th and 18th passages were found to be infectious for rabbits.

Numerous attempts were made to adapt the virus to growth on the chorioallantoic membrane. In each case where virus from fresh tissues was employed it multiplied in the egg. However glycerinated virus did not always establish itself. In some cases the virus passage was discontinued after only 3 or 4 transfers, in others it was carried through 10 or 12 transfers. It was found that the size of the inoculum could be varied between 0.1 and 0.35 cc, the membranes could be ground in physiological saline, saline buffered with phosphate, veal infusion broth, or even egg fluid, though the latter was less satisfactory. It was, however, found necessary to make passages quite regularly at 3-day intervals. The titer of virus was lower on the 2nd and 4th days than on the 3rd, so that passage at 4-day intervals resulted in loss of the virus within 2 to 3 passages. Eggs which had been incubated for 11 days prior to inoculation sustained the growth of the virus most satisfactorily, regularly yielding membranes which were infectious for rabbits when

[5] Faulkner, G. H., and Andrewes, C. H., *Brit. J. Exp. Path.*, 1935, **16**, 271.

[6] Paschen, E., *Zent. Bakt.*, 1936, **138**, 1.

[7] Shope, R. E., *J. Exp. Med.*, 1932, **56**, 803.

diluted 10^{-4} or 10^{-5} by weight.† The titers obtained with 9-day eggs were slightly lower and the titers with 13-day eggs approximately 100-fold lower.

In later passages, whenever possible contamination was feared, penicillin (165 units per egg) and streptomycin (0.0015 g per egg) were added to the membrane suspension.

Viability of Egg Passage Virus. Suspensions of membranes made up in veal infusion broth pH 7.3 to which 20% rabbit serum was added were found to withstand quick freezing with dry ice and alcohol and storage in the dry ice chest for at least 2 months. Suspensions in saline, in egg fluid or even in plain broth were often inactive on thawing. Virus suspensions allowed to stand overnight in the refrigerator showed a roughly 100-fold fall in titer.

Distribution of Virus within the Egg. On several occasions the embryos, yolk sacs, and pooled egg fluids from infected eggs were tested for virus by subcutaneous inoculation into a rabbit. Even when the chorioallantoic membrane suspension proved infectious to rabbits when diluted 10^{-4} or 10^{-5}, no virus was detectable in any other part of the egg.

Routes of Inoculation. Virus which had been through a number of passages on the chorioallantoic membrane was injected in 0.5 cc amounts into the yolk sac. Embryos and yolk sacs harvested 3 to 5 days later contained no demonstrable virus.

Effect of Virus upon the Chick Embryo and its Membranes. In view of the widespread lesions produced on the chorioallantoic membrane by the related myxoma virus,[8,9] it was a disappointment to find no consistent and recognizable effect of fibroma virus on the chick embryo. Many membranes which were shown by rabbit inoculation to contain virus in large amounts appeared as delicate in structure as membranes from normal eggs. On the other hand it seemed, in the course of adapting virus from a number of individual rabbits to growth in the egg, as if the strains derived from some rabbits showed more tendency to cause marked edema of the membrane than did other strains. Passages were made in parallel from edematous and from "non-edematous" membranes. Sometimes the edema-producing capacity would remain for a passage or two before fading out. Once it had disappeared it would not reappear in the course of further passages. The nature of this "lesion" is being subjected to further study at the present time.

Sections of a number of membranes were studied histologically. The spindle shaped cells with large nuclei characteristic of fibroma in the rabbit[3,10,11] could not be demonstrated in the egg. No inclusion bodies were seen. Contrary to the experience reported by Paschen[6] we were unable to demonstrate elementary bodies with certainty on smears stained with Morosow's stain.

The embryos of eggs infected with fibroma virus appeared normal. Several batches of infected eggs were allowed to hatch. The chicks from these eggs were not tested for presence of virus; however, serum obtained from them at the age of 15 days after hatching failed to neutralize rabbit passage fibroma virus. This lack of detectable immunologic response may well be due to the well-known fact that immature animals respond poorly to antigenic stimuli.

Demonstration of Presence of Virus in the Egg. In the absence of characteristic lesions, the only means of recognizing the presence of virus was back-passage into the rabbit. Quicker methods of recognizing infection in the egg were tried unsuccessfully. The supernates of infected membrane suspensions, both unheated and heated for 40 minutes at 70°C[12]

† Tumor tissue or testicle removed from a rabbit at the height of infection, *i.e.* about 6 days after inoculation, is usually infectious when diluted 10^{-5} or even 10^{-6}. Thus the titer obtained with egg passage virus even under optimal conditions is not quite so high as in the case of rabbit passage virus.

8 Lush, D., *Australian J. Exp. Biol. and Med. Sc.*, 1937, **15**, 131.

9 Haagen, E., and Du Dscheng-Hsing, *Zentr. Bakt.*, 1938-39, **143**, 23.

10 Hurst, E. W., *Australian J. Exp. Biol. and Med. Sc.*, 1938, **16**, 205.

11 Ahlstrom, C. G., *J. Path. and Bact.*, 1938, **46**, 461.

12 Mills, K. C., and Dochez, A. R., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 140.

were tested for hemagglutination against red cells from the chicken, duck, mouse, guinea pig, rabbit, horse and sheep; but the membranes from infected eggs produced hemagglutination no more regularly than did those of the uninfected eggs used as controls. Precipitin tests and skin tests in rabbits, using as antigen the allantoic fluid of infected eggs yielded negative findings.

Identification of the Virus Obtained upon Egg Passage. That the virus passed in the egg was actually fibroma virus was shown in 3 ways.

(a) Rabbits inoculated on different occasions with egg passage virus were challenged 2 to 3 weeks later with glycerinated rabbit passage virus and to this they were found to be immune.

(b) A rabbit was immunized by subcutaneous and intratesticular inoculation of egg passage virus and another rabbit was similarly immunized with rabbit passage virus. Both were bled after 14 days and their respective sera used in a neutralization test against fresh rabbit passage virus. Both sera neutralized the virus, no lesion appearing where the mixture was inoculated, whereas areas injected with control mixtures of virus and normal rabbit and virus and saline displayed tumor formation.

(c) A rabbit injected with 8th egg passage fibroma virus was challenged 6 weeks later with a subcutaneous injection of rabbit passage myxoma virus. A control rabbit receiving the same inoculum was dead of myxoma in 10 days, whereas the rabbit which had previously been exposed to egg passage fibroma virus showed no signs of illness. In view of the regularity with which myxoma, virus causes death in normal rabbits it seems certain that the inoculation with egg passage fibroma virus had protected the rabbit against an otherwise fatal infection with myxoma, a phenomenon first described by Shope[7] in connection with rabbit fibroma.

Growth of the IA Strain of Virus in the Egg. Only 2 experiments were carried out with the IA strain of virus. In both cases the virus was lost at the 4th passage, owing to bacterial contamination. No lesions were observed in the infected eggs. In the one case in which the virus titer was determined it was only 10^{-2} at the 3rd passage.

Discussion. The experiments described here show that at least one strain (the OA strain) of the virus of infectious fibroma of rabbits can readily be propagated on the chorioallantoic membranes of the developing hen's egg. Although the titers of virus obtained from infected membranes compared favorably with these from rabbit tumor tissue, the virus did not, in our experience, show any tendency to invade the embryo and was not demonstrable in the extra-embryonic fluids. Likewise when virus propagated for a number of passages on the chorioallantoic membrane was injected into the yolk sac it rapidly disappeared. It is possible that virus which had been subjected to a greater number of passages on the chorioallantoic membrane might display greater invasiveness for the egg.

Passage through the embryonated egg did not seem to alter the type of lesion for its original rabbit host. This finding was rather unexpected, in view of the fact that the virus did not produce in the egg the pathological changes which are characteristic in the rabbit, *i.e.* intense fibroblastic proliferation with production of tumor-like masses and, in the wild cottontail rabbit, very striking cytoplasmic inclusion bodies in the overlying epithelium. No such bodies could be found in epithelium covering the chorioallantoic membrane.

Summary. 1. The virus which is the causative agent of infectious fibroma, giving rise to tumor-like nodules in rabbits, was maintained by serial passage on the chorioallantoic membrane of the embryonated hen's egg for 18 consecutive passages.

2. The titer of virus in infected chorioallantoic tissue was slightly lower than the titer in rabbit tissues. It was maintained by using eggs incubated for 11 days prior to inoculation and making passages at 3-day intervals.

3. Invasion of the embryo and extra-embryonic fluids did not take place in eggs inoculated on the choriallantoic membrane. Inoculation of the virus into the allantoic sac, the amniotic sac, the yolk sac and the embryo itself yielded negative results.

4. Although marked edema of the chorioallantoic membrane was noted in some of the infected, no pathognomonic lesion, gross or microscopic, was present, so that it was necessary in every instance to resort to backpassage into rabbits to demonstrate the presence of virus.

5. The type of lesion produced in rabbits by the virus was not significantly altered by repeated passage in hen's eggs.

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Effect of Cholecystectomy on Fecal Fat Excretion in Dogs.*

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In a previous report[1] it was demonstrated that diversion of bile from the intestine in dogs results in the failure of absorption of about 60% of fat ingested in the diet. It was also found that oral administration of various bile salt preparations failed to correct this steatorrhea.[2] One of the questions raised by these results concerned the possible role in fat absorption of the manner of entry of bile into the intestine. It was of interest, therefore, to determine whether cholecystecomy, which converts the normal intermittent flow of concentrated bile into a continuous flow of dilute bile into the intestine,[3] would result in a defect in fat absorption.

Methods. Eight normal dogs were placed on a daily diet of 335 g of "Pard" with 40 g added lard, making a total fat content of 53 g. After two days on the diet, feces were collected over a 10-day period and pooled for analyses of total fat and free fatty acids[4] and for nitrogen (Kjeldahl). The animals were then cholecystectomized and allowed 2 weeks for recovery. Four animals were then replaced on the original diet while the other 4 were given a higher fat diet consisting of 200 g "Pard" with 70 grams added lard (total fat content, 80 g). Feces were again collected over the last 10 days of a 12-day period, and analyzed. This procedure was next repeated, interchanging the diet for the two groups of dogs. Finally the dogs were sacrificed and autopsy revealed complete removal of the gallbladder and moderate dilation of the biliary passages.

Results. The mean daily excretions of fat and nitrogen in the feces of 8 dogs both before and after cholecystectomy are presented in Table I. The average total fat excretion of 2.43 g per day compares fairly well with the previously reported value of 3.51 g obtained from 5-day tests on 6 dogs and demonstrated to be independent of the fat content of the diet.[1] Cholecystectomy did not significantly alter this rate of fat excretion. Following the operation the percentage of split fecal fat significantly increased; the meaning of this is obscure since further analysis showed that it occurred only during the first experimental period following cholecystectomy. Fecal nitrogen excretion was unaffected by cholecystectomy, although the lower "Pard" content of the high fat diet resulted in diminished nitrogen excretion, due as previously suggested[1] to the reduced content of crude fiber.

Discussion. The present results clearly indicate that there is no impairment in the absorption of dietary fat or nitrogen subsequent to a change from an intermittent flow of concentrated bile to a continuous flow of dilute bile into the intestine. These results confirm a previous study in which 6 dogs were given

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1 Heersma, J. R., and Annegers, J. H., *Am. J. Physiol.*, 1948, in press.

2 Heersma, J. R., and Annegers, J. H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 339.

3 Puestow, C. B., *Arch. Surg.*, 1931, **23**, 1013.

4 Fowweather, F. S., and Anderson, W. N., *Biochem. J.*, 1946, **40**, 350.