we have seen from the control growth that an input multiplicity of at least 0.117 or 3.5×10^5 VP is needed to bring 3 million L cells to the observed yield of 7×10^9 VP in 7 days. Clearly there must have been cell infection and virus increase in the blank areas of the plaque plates. Furthermore, this increase, from ⅔ of 199 to 3.5×10^5, would mean that every particle infected a cell and each cell produced 2650 VP. However, one might expect some incompleteness in the collection of infected cells from the original monolayer under the agar as well as less than 100% adsorption of virus to the cells of the test cultures. These would raise the estimate for VP production in the few infected cells under the agar above 2650 per cell but not necessarily to a figure greater than those indicated in the control cultures of Fig. 1. It is therefore possible that all of the 199 VP that did not produce plaques, did infect one cell each and these provided the minimum 3.5×10^5 VP necessary to bring the bottle cultures to full yield. It is, of course, possible that the yield of virus from these few initially infected cells was not so great but that a few adjacent cells became infected, producing a microplaque and yielding the observed number of virus particles.

On further passage, the virus recovered from the blank areas between the plaques has shown the same efficiency of plaque formation as that recovered from the plaque area. Why, then, did it not make plaques on the initial monolayer? The population of L cells has given evidence of uniformity in VP yield per cell when inoculated at an input multiplicity of 10(2) but there is no data yet that shows uniformity of yield from singly infected individual cells. It is possible that a few cells, perhaps 1 in 200 in these experiments, yield a superior number of VP, a number sufficient to infect several adjacent cells and produce a visible plaque while the large majority of infected cells do not provide this critical number.

Summary. Although only a few plaques may appear when many vaccinia virus particles are put upon a monolayer of L cells, many cells become infected in areas remote from the plaques. A large fraction of the particles of the inoculum do infect cells and harvest of these from "blank" areas shows that substantial virus growth has occurred.

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Absorption of Taurocholate-24-^{14}C Through the Canine Gastric Mucosa.* (32176)

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When the gastric mucosa is damaged by salicylates or fatty acids it becomes abnormally permeable to H^+, Na^+, K^+, glucose, histamine and plasma proteins(1). In the course of unpublished work on the effects of natural and synthetic detergents upon the barrier function of the gastric mucosa, irrigation of the mucosa with neutral or acid solutions of conjugated bile salts was found to increase the mucosa's permeability. The possibility that the abnormally permeable mucosa permits bile salts to pass from gastric

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lumen to blood was tested in the experiments described here.

Methods. The dogs and the method of irrigating their pouches have been described in detail(2). Briefly, unanesthetized dogs in good health, each having a separated vagally denervated (Heidenhain) pouch of the oxyntic gland area of the stomach, were used. At the beginning of each experiment the pouch was washed thoroughly with 154 mM NaCl and then once with the solution to be used. The pouch was then filled with a taurocholate solution which was well mixed in the pouch and then sampled. The volume of the sample was measured and its radioactivity determined. The initial volume in the pouch after sampling was known and was between 33 and 36 ml. Sixty minutes later the fluid in the pouch was removed and measured, and its radioactivity was determined.

Two solutions of bile salts were used. Gall-bladder bile was obtained from dogs at the end of acute experiments, pooled, analysed for total bile salts concentration by the method of Wheeler and Ramos(3) and kept frozen until needed. The quantity required to make a solution 10, 20 or 40 mM in bile salts was placed in a 100 ml volumetric flask; 10 ml of 150 mM Na phosphate buffer, pH 7.4, and 0.2 ml of a solution of chromatographically pure Na taurocholate-24-^{14}C (Tracerlab) were added; and the solution was made up to volume with 154 NaCl. For other experiments pure synthetic Na taurocholate (Maybridge) in quantities required to make a 10, 20 or 40 mM solution was placed in a 100 ml volumetric flask and made up in the same way.

For counting, 0.1 ml of each sample was pipetted into a vial to which was added 15 ml of the standard dioxane-PPO-POPOP scintillation solution. Samples in duplicate plus background blanks and quenched standards were counted in a Nuclear-Chicago scintillation counter. Counting of the irragation samples was done for 20 minutes, and total counts in the experimental vials was of the order of 10,000 counts. Corrections for efficiency of counting were made by the 2 channel method. Efficiency was in the range of 68-72% and did not vary more than 2% during counting runs. Efficiency of counting bile obtained by cannulation of the bile duct was close to 50%. Results are reported in disintegrations per minutes (dpm). Disappearance of radioactivity from the pouches was calculated by subtracting the product of the initial volume and the initial dpm per ml from the product of final volume and final dpm per ml; the result is negative indicating net flux out of the lumen. Taurocholate disappearance was calculated by dividing dpm disappearance by the specific activity of the taurocholate solution used. Since taurocholate as such was not determined in gall-bladder bile, the results of experiments with natural bile solutions are in error to the extent that other bile salts were included in the total bile salt concentration.

Each solution was used twice on each of 2 dogs with at least 48 hours between experiments.

Results. Results of irrigation experiments are given in Table I. In all instances total radioactivity recovered from the pouches at the end of 60 minutes was less than that initially instilled. Calculated rate of disappearance of taurocholate was greater the higher the concentration of taurocholate in the irrigation solution.

These data show that taurocholate disappeared from the lumen of the gastric pouch; that it was actually translocated to venous blood was demonstrated by the following experiment. A dog was anesthetized with pentobarbital; its bile duct was cannulated; and its cystic duct was ligated. An intravenous infusion of non-radioactive, synthetic taurocholate was started at the rate of 11.5 μM per minute and continued for the duration of the experiment. A perforated plastic tube passing through a plastic spool(4) was inserted into the stomach through an incision in the terminal antrum. The antral wall was tied around the spool in such a manner that there was no possibility of leakage of gastric contents into the small intestine. The stomach was washed out with 154 mM NaCl and filled with 100 ml of the same solution. Bile was collected for 3 15-minute periods. The NaCl solution was then replaced by 100 ml of a solution of 10 mM gall-bladder bile in 15 mM Na phosphate buffer, pH 7.4, and 154 mM

NaCl. The bile solution was labeled with Na taurocholate-24-^{14}C so that its activity was 1.25×10^5 dpm per ml. Precautions were taken to prevent any contamination of bile collected by the solution instilled into the stomach. Bile was collected for 8 successive 15 min periods, and then the stomach was emptied, washed 6 times with 154 mM NaCl and filled with 100 ml of the NaCl solution. Radioactivity of bile collected was measured as described above. Results are given in Fig. 1.

Discussion. Bile salts are actively absorbed in the ileum(5), and although they enter the cells of the upper small intestine(6), they are poorly if at all translocated from lumen to blood in the duodenum or jejunum. The data presented here show that taurocholate does pass through the mucosa of the gastric oxyntic gland area and that it reaches the liver from the stomach. Since bile salts in the stomach increase the permeability of the gastric mucosa as judged by fluxes of H^+ and Na^+ across it (unpublished work), it is more likely

TABLE I. Absorption of Taurocholate-24-^{14}C from Pouches of Oxyntic Gland Area of Two Dogs.

Dog	[Bile salt], μM/ml	SA,* dpm/μM	Δ dpm/hr	Δ Taurocholate, μM/hr
	In solution of gall-bladder bile			
1	10	2,351	—67,295	—28
	"	968	—26,500	—27
	20	495	—16,282	—33
	"	484	—24,223	—50
	40	215	—18,827	—88
	"	238	—16,281	—68
2	10	2,313	—97,378	—42
	"	937	—18,000	—19
	20	513	—39,858	—78
	"	483	—28,953	—60
	40	211	—33,305	—158
	"	229	—13,906	—61
	In solution of synthetic taurocholate			
1	10	871	—35,961	—41
	"	973	—19,342	—20
	20	503	—34,189	—68
	"	471	—28,304	—60
	40	284	—86,391	—304
	"	234	—25,225	—108
2	10	869	—25,638	—30
	"	912	—10,683	—12
	20	481	—28,564	—59
	"	465	—48,128	—104
	40	236	—13,927	—59
	"	232	—47,825	—206

* Specific activity.

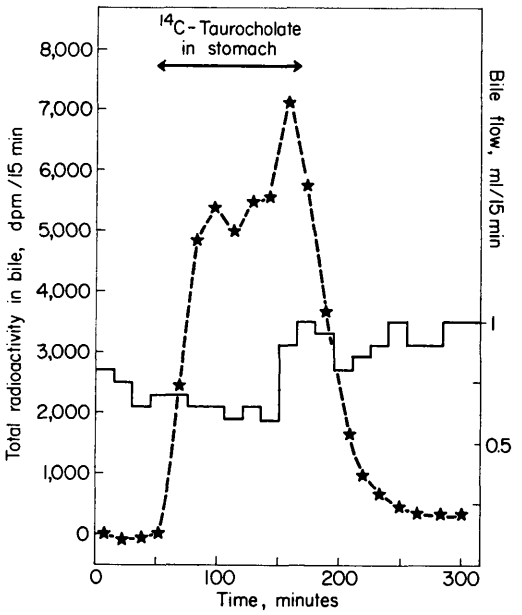

FIG. 1. Bile flow rate (horizontal lines) and ^{14}C-radioactivity (points and broken line) of bile of anesthetized dog over 315 min. At 48 min stomach was filled with 100 ml isotonic, neutral, buffered solution of dog bile (10 mM total bile salt concentration) labeled with taurocholate-24-^{14}C to 1.25 x 10^5 dpm per ml. At 166 min labeled bile solution was removed from stomach, and stomach was washed out with 154 mM NaCl.

that bile salt absorption from the stomach is the result of passive diffusion through relatively large pores than of active absorption.

Summary. When neutral solutions of natural dog bile or of synthetic taurocholate labeled with chromatographically pure taurocholate-24-^{14}C were placed in pouches of the oxyntic gland area of the dog's stomach, total radioactivity decreased. Calculated rate of absorption of taurocholate was greater the higher the concentration of taurocholate in the pouch, and it ranged from 12 to 304 μM per hour. That bile salt can be absorbed from the stomach into venous blood was shown by the fact that when the stomach of an anesthetized dog was filled with a neutral solution of 10 mM bile salts derived from dog bile and labeled with taurocholate-24-^{14}C, radioactivity appeared in the fluid collected by cannulation of the animal's bile duct.

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Anti-Granuloma and Anti-Edema Properties of Corticosterone in Hyper- and Hypothyroid Rats. (32177)

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Hypothyroidism may reduce anti-inflammatory properties of glucocorticoids(1,2), although this evidence has been challenged (3,4). Thyroid hormones may increase thymus weight(5) and aggravate hypersensitivity reactions to egg white or dextran(6,7), yet hyperthyroidism is accompanied by ACTH-release(5,8), adrenal hypertrophy and elevated plasma corticosteroids(8)—events which would seemingly favor reduced inflammatory potential and thymolysis.

In an attempt to resolve these conflicting views, antigranuloma, anti-edema and thymolytic properties of corticosterone were studied simultaneously in hyper-, hypo- and euthyroid rats. Participation of endogenous hormones was evaluated by comparing intact, thyroidectomized, adrenalectomized and hypophysectomized rats.

Materials and methods. One hundred and sixty-two male rats (CD® strain) weighing 120-130 g initially were purchased from Charles River Breeding Laboratories in 4 basic groups: intact, thyroidectomized ("TX"), adrenalectomized ("ADX", salt maintained) and hypophysectomized ("HYPOX").* Two and one-half weeks after operation, each basic group was divided into 4 subgroups (10-11 rats each) and treated as follows: 1. no treatment (control); 2. corticosterone, 30 mg/kg s.c. in 0.2 ml sesame oil daily; 3. thyroglobulin (Proloid® W1744, Lot EM-3) at level (300 μg % iodine) equivalent to 0.15% USP thyroid in ground chow diet; 4. combination of corticosterone injections and thyroid diet. Treatments were continued for 9 days. Two cotton pellets (35 mg each) were implanted s.c. on day 1. On day 8, each rat received plantar injections of 0.1 ml 0.85% saline (right front paw) and 0.1 ml 1% ovalbumin (left front paw). At 4 hours circumference of control (saline) and edematous (ovalbumin) paws were compared and results expressed as the difference $\pm$ SE. On day 9, rats were killed with ether and cotton pellet granulomas (after oven drying to constant weight), adrenals and thymus were weighed to the nearest 0.1 mg. Results were presented as mean values $\pm$ SE and probability of significance of differences was determined in Fischer's "t" table.

Results. Despite large differences in body weight gain, dry granuloma weight was similar in intact, Tx, Adx or Hypox control rats, and corticosterone inhibited granuloma formation similarly in each of these groups (Table I). Thyroid feeding depressed granuloma weight in Tx rats, but did not influence granuloma formation in other groups nor modify effects of corticosterone on granulomas (Table I). Increases in circumference of edematous paws after egg white injection were similar to control in Adx or Hypox rats but less ($p<0.05$) in Tx rats (Table I). Corticosterone partially prevented circumferential increase in edematous paws of intact,

* Completeness of hypophysectomy was checked at autopsy by exploration of the pituitary area for possible remnants and by observation of testicular size.