tered as a freshly made preparation but toleration declined to 69% when emulsions were 5 to 10 weeks old. A significant rise in concentration of free fatty acids and unstable peroxides or increase in size of fat particles up to 25 μ has been described as characteristic of aging emulsion. Accumulation of any of the latter products might be related to toxicity and thermogenicity of the emulsion when used for intravenous alimentation. These reactions may be compared to toxic effects produced experimentally by injection of free short chain fatty acids(5) or to pulmonary and pyrogenic effects associated with trapping of particulate matter in the lungs(6). Stability of an artificial emulsion should be considered to be a function of time during which physical alterations in size of fat particles and chemical interactions between water and fat may occur. A technic for rapid emulsification of oil and water mixtures with elimination of the factor of aging would therefore be warranted. Although the present "instant" fat emulsion cannot now be considered to be a suitable preparation for general clinical use, investigations along the lines described in this report have been continued.

Summary. An "instant" fat emulsion prepared by manual mixing of oil, emulsifier, alcohol and water was developed and tested. This type of emulsion obviated high pressure homogenization and was freshly prepared for each infusion. A total of 63% of the infusions were fairly well tolerated while in the other 37% reactions were observed.

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Modification of Tumor-Response to Rous Sarcoma Virus (RSV) by Hydrocortisone.* (22184)

VINCENT GROUPÉ, FRANK J. RAUSCHER, AND W. RAY BRYAN.

Institute of Microbiology, Rutgers University, State University of N. J., New Brunswick and National Cancer Institute, National Institutes of Health,† Bethesda, Md.

It is well known(1) that cortisone and hydrocortisone usually depress resistance of laboratory animals to viral infection and that effective doses of these steroids are frequently associated with evidence of increased multiplication of the virus. On the other hand, cortisone and several related steroids beneficially affect certain transplanted lymphosarcomas and leukemias in mice(2). However, rabbits injected with cortisone developed much larger tumors upon inoculation with rabbit fibroma virus although tumor size was not appreciably affected if injections of cortisone were delayed until appearance of the tumor(3). Data presented here show that (a) when Rous sarcoma virus (RSV) was inoculated into chicks pretreated with large doses of hydrocortisone, tumors appeared later and were greatly altered in appearance; (b) when hydrocortisone was discontinued the altered tumors promptly reverted to typical invasive type; and (c) when lower doses of hydrocortisone were employed or when injections of hydrocortisone were delayed until 2 days after in-

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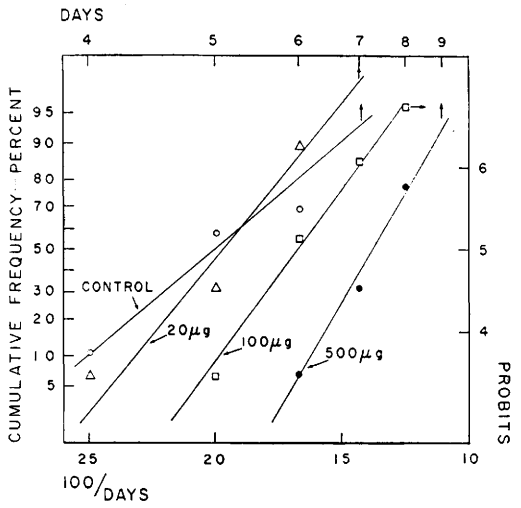

FIG. 1. Effect of pretreatment with hydrocortisone on latent period of RSV.

oculation of RSV the latent period was not affected; tumors which appeared were typically invasive but increased in size at a rate which was appreciably more rapid than that of control chicks.

Materials and methods. RSV (also referred to as chicken tumor I agent) was maintained by serial passage in chickens. A single lot (CT 581) of frozen standard RSV, prepared by differential centrifugation and preserved at –70°C as previously described (4), was used throughout. The diluent for standard RSV was saline containing 2% inactivated (56°C for 30 minutes) normal horse serum. Unsexed white Leghorn chicks under 7 days of age were used. Two tenths ml of suitably diluted RSV were inoculated into the subcutaneous tissue of left wing web. Ventral surface of the wing was swabbed with isopropanol and the hypodermic needle was inserted through the muscle into subcutaneous tissue of wing web, thus avoiding leakage. Each bird was examined daily by swabbing the wing with isopropanol and examining the wing with both direct and transmitted illumination. Hydrocortisone was suspended in the usual diluent(5) and 0.5 ml of the suspension were injected intramuscularly into the thigh muscle. Control birds received daily injections of diluent alone. Data were analyzed as previously described(6,7) using probit analysis(8).

Results. Fig. 1 shows effect of various doses of hydrocortisone on latent period of chicks inoculated into the wing web with RSV diluted 10^{-2}. Daily intramuscular injections of hydrocortisone were begun 2 days before inoculation of RSV and continued throughout the experiment. Control and treated groups consisted of 33 chicks each. It is clear that the latent period was prolonged in birds injected daily with 500 or 100 μg of hydrocortisone. However, the latent period in birds injected with 20 μg of hydrocortisone was not affected, but tumors which appeared were much larger than those in control birds.

In the above experiment the most prominent feature observed was the fact that all tumors which appeared in birds injected with 500 μg of hydrocortisone were grossly, markedly altered in appearance. In contrast to the usual rapidly growing, soft invasive tumor, the tumors which arose in hydrocortisone-treated birds were firm, sharply circumscribed and grew much more slowly. Characteristics of these altered tumors are summarized in Table I and photographs of typical and altered tumors are shown in Fig. 2-4.

Table II summarizes data showing the effect of daily dosage and dosage schedule on latent period and incidence of altered tumors. Altered tumors were produced only in chicks previously injected with large doses (500 μg/chick, *i.e.* 10 mg/kg) of hydrocortisone. Indeed, when dosage was decreased or delayed until after inoculation of RSV the tumors were not only typical and invasive but also were obviously larger and grew more rapidly

TABLE I. Characteristics of Tumors Produced by RSV* in Hydrocortisone-Treated and Control Chicks.

Characteristic	Control	Hydrocortisone-treated
Latent period†	4.6 days	7.6 days
Site of developing tumor	Wing web	Muscle
Type	Multiple	Single
Rate of growth	Rapid	Slow
Type of "	Diffuse & invasive	Spherical, sharply circumscribed
Consistency	Soft	Firm

* Standard RSV diluted 10^{-3}.
† LP_{50} or time in days to produce tumors in 50% of the chicks.

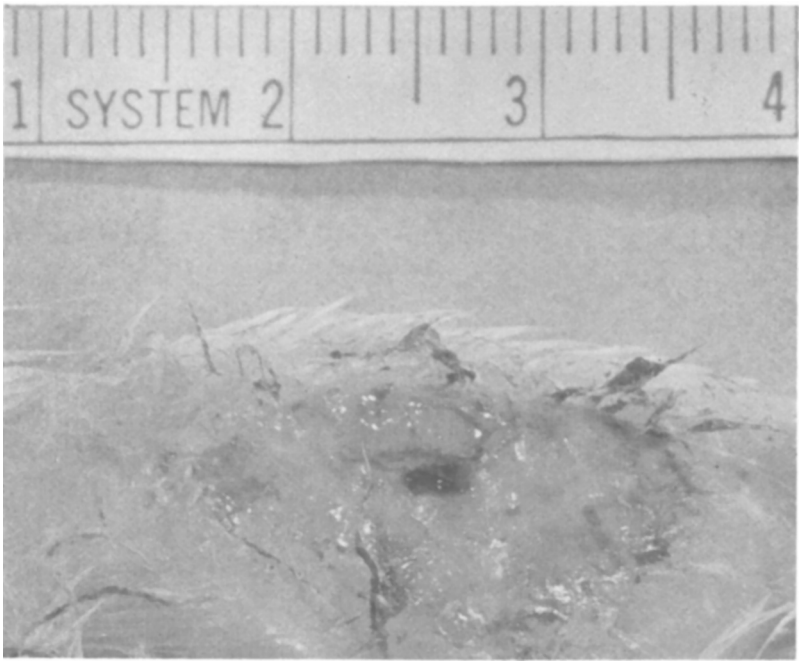

FIG. 2. Control tumor in wing. Twenty-one days after inoculation of RSV diluted 10^{-1}. Scale metric system.

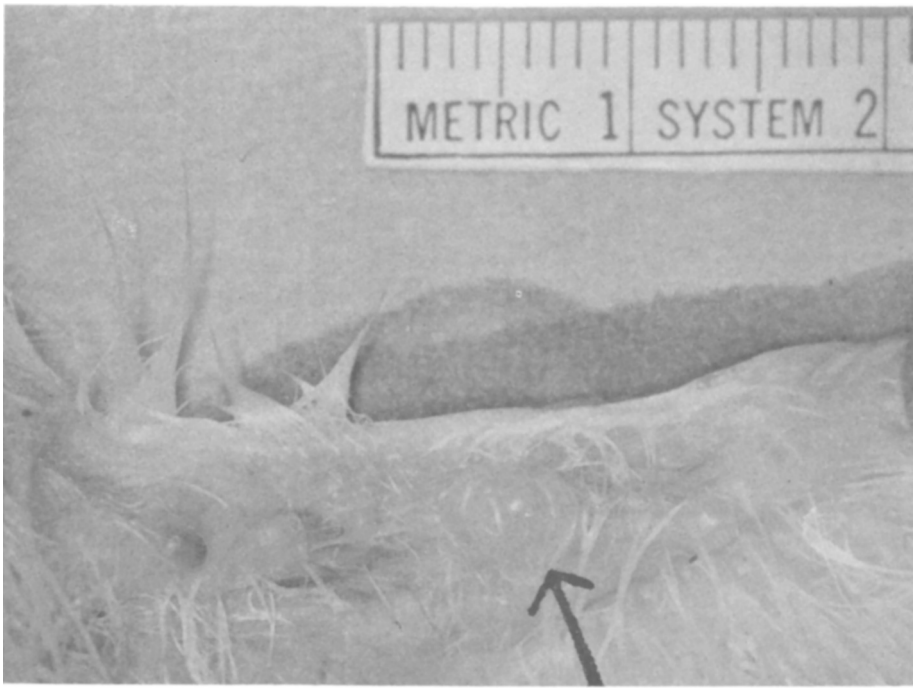

FIG. 3. Altered tumor produced by pretreatment with hydrocortisone. Front (ventral) view. Twenty-one days after inoculation of RSV diluted 10^{-1}.

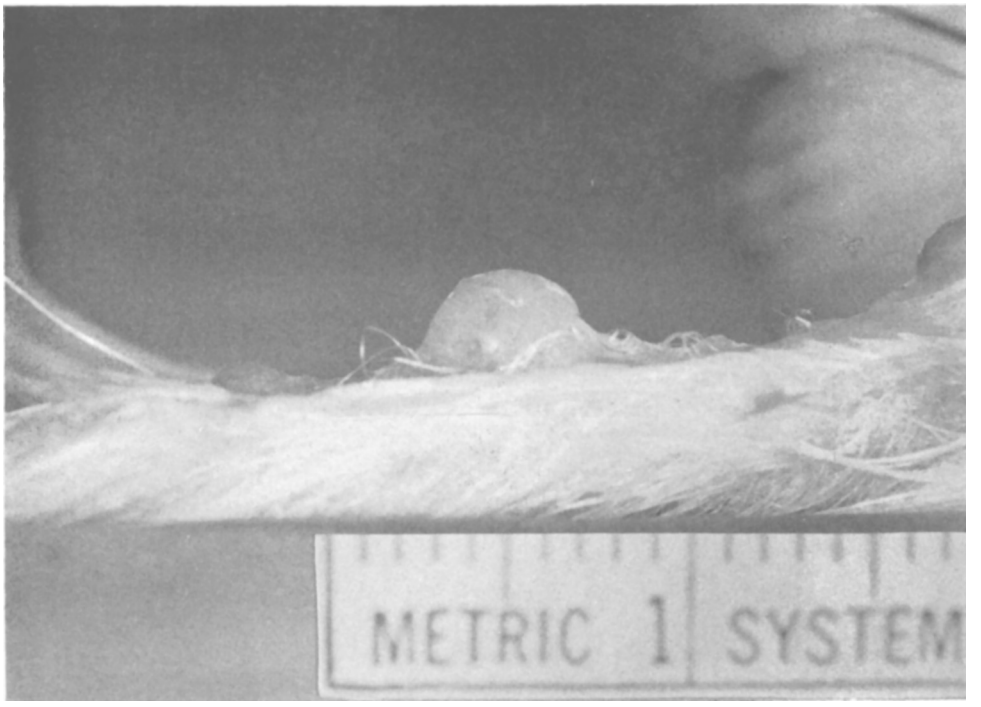

FIG. 4. Side view of altered tumor shown in Fig. 3.

than tumors in control birds.

Fig. 5 shows that when daily injections of hydrocortisone were discontinued almost all altered tumors promptly reverted to typical invasive tumors. In this experiment, 2 groups of 34 chicks each were injected daily with 500 μg of hydrocortisone and diluent, respectively, beginning 2 days before inoculation of RSV diluted 10^{-2}. All tumors in the treated group appeared as altered tumors and all control tumors were typical and invasive. On 10th day after inoculation of RSV the treated group was divided in half and injections of hydrocortisone were discontinued in one subgroup and continued in the other. It is clear (Fig. 5) that all but one of the altered tumors promptly reverted to typical invasive tumors in the subgroup in which hydrocortisone was discontinued and the tumors remained altered in those birds which continued to be injected with hydrocortisone. The single bird with an altered tumor which failed to revert when

TABLE II. Modification of Tumor-Response to RSV by Hydrocortisone.

Daily intramusc. inj. of hydrocortisone			Type of tumor:		
Begun on day:	Daily dosage, μg	No. of chicks	Typical, %	Altered, %	Latent period LP_{50}, days
−2	Diluent	30	100	0	4.3
"	20	"	100	0	4.2
"	100	"	90	10	5.3
"	500	"	0	100	6.7
"	Diluent	35	100	0	4.6
+2	500	"	100	0	4.5
0	"	"	97	3	5.4
−2	"	"	0	100	7.6

LP_{50} = Time in days to produce tumors in 50% of birds.
−2 = 2 days before RSV; +2 = 2 days after RSV.
RSV diluted 10^{-2}.

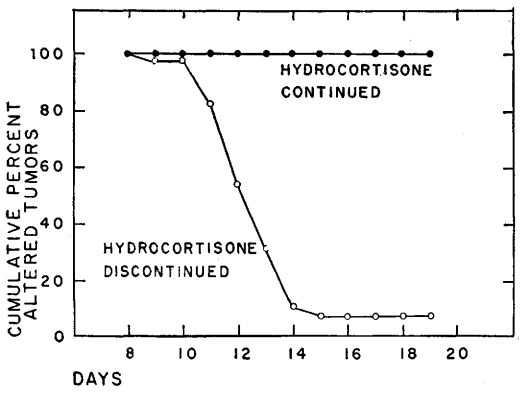

FIG. 5. Reversion of altered tumors after discontinuance of hydrocortisone.

hydrocortisone was discontinued, was also unusual in that the latent period of the altered tumor was unusually prolonged (10 days) by hydrocortisone.

In the single experiment described below the virus content of tumors altered by hydrocortisone was compared with that of control tumors. Groups of 30 chicks each were injected daily with 500 μg of hydrocortisone and diluent alone beginning 2 days before inoculation of RSV diluted 10^{-1}. Eleven days after inoculation of RSV, 6 birds from the control group and 11 from the treated groups were sacrificed and their tumors were removed aseptically, pooled, and stored in glass sealed tubes at −70°C. Potencies of the 2 pools of tumor tissue were compared with one another and with standard virus as previously described(9). The data indicated that the virus content of altered tumor tissue was lower than that of the control (typical) tumor tissue by a factor of approximately 10.

Discussion. Tumor-response to RSV was strikingly different in chicks that were pretreated with hydrocortisone. This modifying effect of hydrocortisone contrasts sharply with the increased rate of tumor growth observed when injections of hydrocortisone were begun after inoculation of RSV. Alteration of tumor-response to RSV by pretreatment of chicks with hydrocortisone is markedly affected by dosage of hydrocortisone employed. Thus, altered tumors were produced in chicks pretreated with large doses of hydrocortisone (10 mg/kg) while pretreatment with low doses (0.4 mg/kg) of hydrocortisone not only did not produce altered tumors but the typical, invasive tumors which arose were substantially larger than the control tumors. Studies on the mechanisms involved in these paradoxical effects are in progress.

Summary. When RSV was inoculated into chicks pretreated with large doses of hydrocortisone the tumors appeared later and were greatly altered in appearance. These altered tumors were sharply circumscribed, firm, whitish and grew more slowly than control tumors. When hydrocortisone was discontinued the altered tumors promptly reverted to typically soft and invasive tumors. When lower doses of hydrocortisone were employed or when injections of hydrocortisone were begun after inoculation of RSV the tumors which appeared were typically invasive and grew more rapidly than the controls.

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