Phatak[2] on isolated intestinal muscle. Divinyl oxide, another of the newer anesthetic agents, was shown by the same authors[3] likewise to increase the tonicity of the isolated intestinal segment. The present study is concerned with the effect of divinyl oxide on intestinal activity *in vivo*.

Six dogs were prepared with Thiry-Vella loops of the upper jejunum. When healing had occurred, tracings of the intestinal movements were recorded after inserting a balloon, connected to a water manometer, into the lumen of the proximal end of the Thiry-Vella loop. Respiratory tracings by means of a pneumograph applied about the chest were simultaneously recorded in order to rule out the possibility that the tracings taken from the balloon were not due to respiratory movements. No preanesthetic medication was administered. All animals were anesthetized with divinyl oxide (Vinethene—Merck) and oxygen by means of a Foregger Metric machine using the closed carbon dioxide-absorption technic.[4] An endotracheal tube fitted with an inflatable cuff was introduced as soon as the state of surgical anesthesia was reached in order to assure a patent airway and thereby obviate the effect of respiratory obstruction and the ensuing anoxemia.

Results. Contrary to the *in vitro* results, all animals showed effects identical to those obtained with ether (di-ethyl oxide); namely, diminished muscular tone and complete inhibition of intestinal contractions during all planes of surgical anesthesia. (Fig. 1.)

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A Fraction from Normal Chick Embryo Similar to the Tumor Producing Fraction of Chicken Tumor I.

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A fraction possessing high tumor-producing activity can be isolated from chicken sarcoma extracts by means of differential cen-

[2] Peoples, S. A., and Phatak, N. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 287.

[3] Peoples, S. A., and Phatak, N. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 378.

[4] Rovenstine, E. A., *Am. J. Surg.*, 1936, **34**, 456.

trifugation at high speed.[1] Application of the same technic to the fractionation of normal chick embryo extracts has resulted in the separation of a fraction presenting similar chemical and physical properties but exhibiting no tumor-producing power.

Eight-day-old chick embryos were used in these experiments. The whole embryo was washed several times in sterile saline solution and then forced through a *Latapie* masher. The resulting watery pulp was frozen at –80°C and stored at that temperature from 3 to 30 days.

The embryo extract was prepared by grinding the frozen tissue with sand and extracting with a phosphate buffer solution.* The suspension was centrifuged at 2400 times gravity for 15 minutes and the deposited tissue was reëxtracted in the same manner. The sediment was discarded and the supernatant fluids from the two extractions were combined and filtered through sterile gauze. The total volume of buffer used for this double extraction corresponded to 15 times the weight of the fresh embryo pulp.

Embryo extracts prepared in this way are opalescent solutions containing about 3.2 mg solids per cc† and having a specific viscosity of about 1.2 as measured in the Ostwald viscosimeter.

Fractionation of this embryo extract by high speed centrifugation was accomplished as follows: The extract was first centrifuged one hour at 17,000 r.p.m. At that speed the corresponding centrifugal force, in the center of the tube, is about 18,000 times that of gravity.‡ The supernatant liquid from this long run at high speed was discarded and the jelly-like sediment was taken up in phosphate buffer solution. This suspension was submitted to a short run of 3 minutes at high speed and the supernatant fluid was saved. The deposit was taken up once more in a small volume of buffer solution and the suspension centrifuged for 3 minutes at high speed. The deposit was discarded and the supernatant fluid combined with that of the previous run. This solution was used for the next series of centrifugation. The whole process consisting in the elimination of the soluble elements of the extract by a long run of one hour at

[1] Claude, A., *Science*, 1938, **87**, 467.

* The extraction, and subsequent washings in the centrifuge, were made with a 0.005M phosphate buffer solution at pH 7.0.

† This figure represents the extracted solids, deduction being made for the salt content from the buffer solution.

‡ The centrifuge used in this work was the Type B, size 1 model, with the multispeed attachment and No. 295 head, of the International Equipment Co., Boston, Mass.

high speed, followed by the removal of the coarse particles by two short runs of 3 minutes, was repeated twice.

During the entire experiment the temperature of the material was maintained near 0°C, except during the long runs at high speed when it attained 12 to 14°C.

The purified fraction isolated by this method forms opalescent solutions in phosphate buffers at pH 7.0. In the dark field microscope§ the material appears to be composed of minute granules similar to those found, under the same conditions, in purified and active chicken tumor preparations. When deposited in the high speed centrifuge, the purified chick embryo fraction appears as a pale yellow or nearly colorless, perfectly transparent, mass. Dried *in vacuo* in the frozen state, the substance is a snow white material which will yield 5% more moisture when heated at 80°C.

Under the above conditions of preparation the fraction separated by high speed centrifugation corresponds, in terms of dry weight, to 5.4% of the original embryo extract, or about 2.9 mg dried substance per gram of fresh embryo pulp.

The behavior of the embryo fraction in acid buffer solutions presents a striking similarity to that of the active fraction isolated by the same technic, from chicken tumor extracts,[1] and the solubility curves obtained in each case are practically identical. Solutions containing 0.17 mg of embryo fraction per cc are completely precipitated between pH 2.2 and 5.0. Beyond these two points, the substance becomes more soluble, and at pH 1.0 and 6.2 respectively, the acid mixtures present the normal opalescence of the neutral solution. The point of maximum precipitation, and probably the isoelectric point, of the material is near pH 3.6.||

Chemical analysis was performed on the purified fraction washed 3 times in cold distilled water, desiccated *in vacuo* in the frozen state, and dried further by keeping the residue 6 hours at 80°C. The results indicate that about 8.5% of the material is nitrogen and 1.8% is phosphorus. About 47% of the dried substance was found to be soluble in ether. The lipoid material can be fractionated into 2 main components, by means of their different solubilities in 80% alcohol. As in the case of the purified tumor fraction the major part of the lipoids from the embryo material presents the properties of lecithin. The less abundant fraction which is soluble

§ We are indebted to Dr. P. Olitsky for the use of the dark field microscope and carbon arc lamp.

|| The hydrogen ion concentration of the mixtures was determined by means of a glass electrode potentiometer.

in chloroform and pyridine, but which is insoluble in other ordinary organic solvents, may belong to the group of cerebrosides.

The portion of the purified fraction which is not soluble in ether contains 16.5% nitrogen and gives positive tests for proteins. The Feulgen test for thymonucleic acid is strongly positive.¶

The absorption spectrum of a freshly prepared solution of the embryo fraction was determined by Dr. A. Rothen. A 0.024% solution of the material in 0.005 M phosphate buffer at pH 7.0 gives a maximum absorption in the wave length region of 2600 Å and the curve is very similar if not identical with that given by certain solutions, at the same concentration, of the purified fraction obtained from active chicken tumor extracts.[2] These results are shown in text Fig. 1.

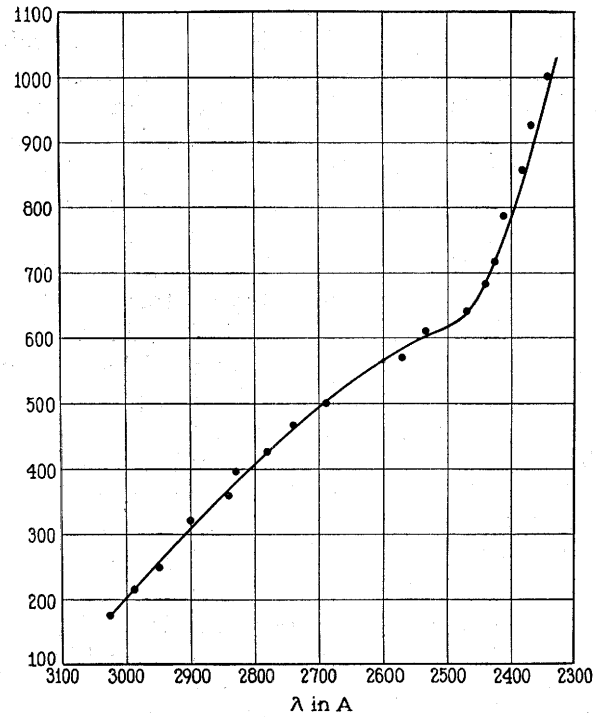

FIG. 1.

Ultraviolet absorption spectrum of the purified fraction prepared from chick embryo tissue by high speed centrifugation. 0.024% solution in 0.005 M. Phosphate buffer, pH 7.0.

¶ Concentrated, purified preparations from chicken tumor I give also a positive test for thymonucleic acid.

2 Claude, A., and Rothen, A., *Am. J. Cancer*, 1936, **26**, 344; Claude, A., *Am. J. Cancer*, 1937, **30**, 742; Claude, A., and Rothen, A., in preparation.

The purified fraction from chick embryo was tested for possible tumor-producing activity by injecting a freshly prepared solution, at various concentrations, into the skin of normal Plymouth Rock hens. The tests have been completely negative, as far as the production of tumors is concerned.

It has been shown that the principle causing a chicken sarcoma can be concentrated in a high speed centrifuge[3] and more recently this method has been utilized to isolate and purify the active material for chemical and physical examination.[1] On dark field microscopic examination this fraction appears to be composed of fine granules, the size of which has been estimated at about 70 mμ in diameter,[4] with a corresponding weight of 2.34×10^{-16} g, as calculated for a density of 1.3.[5] Several workers are inclined to consider these granules as representing the actual tumor agent.[6]

In the present investigation it is demonstrated that chick embryo tissue extracted and centrifuged in the same manner will yield a fraction which, in its main physical and chemical characteristics, is very similar to the active material from the chicken tumor but without tumor-producing properties. The estimated size of the particles are practically the same and the dark field microscopic examination shows the deposit to be made up of the same type of granules.

The purified fraction represents 3.5% of the original tumor extract against 5.4% for the chick embryo extract. Both substances, either from chick embryo or from chicken tumor, have the same solubility in acid solutions, they absorb ultraviolet light in the same manner and both are made up of lipoids and proteins. Color tests and absorption spectrum analysis indicate that one of the main compounds is a nucleoprotein. However, it will be noted that the ether-insoluble portion of the chicken tumor has a nitrogen content of 13.11% while the corresponding fraction of the embryo has 16.5%. It is possible that this difference may be significant. These findings raise the question whether the main constituents of the purified chicken tumor fraction represent inert elements existing also in normal cells or whether the substance found in normal chick embryo tissue may represent a precursor of the chicken tumor principle which could assume, under certain conditions, the self-perpetuating properties of the tumor agent.

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[3] Ledingham, J. C. G., and Gye, W. E., *Lancet*, 1935, **1**, 376; McIntosh, J., *J. Path. and Bact.*, 1935, **41**, 215.

[4] Elford, W. J., and Andrewes, C. H., *Brit. J. Exp. Path.*, 1936, **17**, 422.

[5] Claude, A., *J. Exp. Med.*, 1937, **66**, 59.

[6] Ledingham, J. C., and Gye, W. E., *Lancet*, 1935, **1**, 376; Amies, C. R., *J. Path. and Bact.*, 1937, **44**, 141.

These observations emphasize the necessity of ascertaining further the rôle played by the two chief components of the tumor fraction, namely the lipoids and the nucleoproteins, in the production of tumors.

Summary. By means of a method of differential centrifugation at high speed, a fraction can be separated from normal chick embryo tissue, which, in its main characteristics, resembles the active fraction isolated from chicken tumor extracts by the same method.

The implications of these observations are discussed.

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Constancy of Urea Clearances in Dogs Following Surgical Anesthesias with Cyclopropane, Ether, and Chloroform.*

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Perusal of the literature reveals no extended or correlated study of the effects of the common anesthetics on normal kidney function. Most of the work has been limited to determination of the rate of urine secretion before and after the various anesthetics.[1-4] Studies by Haines, *et al.*,[5] on the effects of ether, morphine, and atropine in various combinations upon dye excretion (phenol red and indigo carmine) before and *during* the period of anesthesia, and of Stehle and Bourne[6] on urine flow, urea, and chloride during and for 3 hours after ether, morphine, or both, have been along similar lines, although with conflicting results. Studies of urea, chloride, phosphate, and water excretion under ether, ethylene, ethylene and amytal, and ethylene and tribromethanol have been carried out by Walton[7] who found no significant changes. Recently Greisheimer, *et al.*,[8] in studies on one dog reported urea clearance elevations of 30%

* Aided in part by a grant from the Wisconsin Alumni Research Foundation.

1 Bonsmann, M. R., *Arch. f. exp. Path. u. Pharm.*, 1930, **156**, 160.

2 Buxton, D., and Levy, A. G., *Brit. M. J.*, 1900, **2**, 833.

3 Kemp, R. C., *New York M. J.*, 1899, **70**, 732.

4 Thompson, W. H., *Brit. M. J.*, 1906, 608 and 667.

5 Haines, W. H., and Milliken, L. F., *J. Urol.*, 1927, **17**, 147.

6 Stehle, R. L., and Bourne, W., *Arch. Int. Med.*, 1928, **42**, 248.

7 Walton, R. P., *J. Pharm. and Exp. Therap.*, 1933, **47**, 141.

8 Greisheimer, E. M., Hafkesbring, R., and Magalhaes, H., *Am. J. Physiol.*, 1938, **123**, 85.