THE INFLUENCE OF VARIOUS SUBSTANCES ON THE GROWTH OF MOUSE CARCINOMA.*[1]

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We carried out the following experiments in order to test the action of various classes of substances on the growth of mouse carcinoma. We used in our experiments a mouse carcinoma, transplanted by us through many generations from a spontaneous tumor sent from Granby, Mass., about seven or eight years ago. In this tumor the cells after a certain initial period of growth are arranged in solid alveoli. Later caseous necrosis sets in in the central parts of the tumor. The tumor was inoculated into American mice weighing approximately sixteen grams. The inoculation was positive in 80 to 90 per cent. (on the average in 82 to 83 per cent.) of the animals. In testing the effect of a certain substance on tumor growth, the mice were injected on the 9th, 10th, 11th, and 12th days after inoculation. The large majority of all injections were done intravenously, the veins of the tail being used. In some cases the effect of subcutaneous injections was compared with the intravenous administration. Two methods were used in determining

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[1] The greater part of this work was carried out during the year 1912 and during the first half of 1913. Since then we enlarged the scope of our work by making additional experiments of a quantitative character. Previous references to these experiments are found in the following communications: Loeb, L., and Fleisher, M. S., *Interstate Med. Jour.*, 1912, xix, 476; 1913, xx, 569; *Jour. Am. Med. Assn.*, 1913, lx, 1857.

In some of our previous papers references have been made to the literature on this subject as far as it preceded our own work. Since we have published our first report a number of communications by various authors have appeared which dealt with the effect of isolated substances rather than with a comparative study of various groups of substances which we undertook. A review of the literature on this subject is given by Keysser, F., *Ztschr. f. Chemotherap., Ref.*, 1914, ii, 1325.

the effect of the injections. (1) On the ninth day after inoculation a drawing of each tumor was made; on the twelfth and thirteenth days another drawing was made. This permitted us to determine with a sufficient degree of certainty whether a tumor had grown very much during this period or whether it had been retarded in its growth. This method was sufficient for determining whether or not a certain substance exerted a decided effect on the tumor growth. The method is inadequate for the determination of finer quantitative differences in the growth of tumors. (2) On the ninth day a drawing of the tumor was made. This drawing was compared with drawings of a series of other tumors of known weight, and thus the approximate weight of the tumor on the ninth day was determined. On the twelfth and thirteenth days further drawings of the tumors were made, and on the thirteenth day the tumors were removed and weighed, either each tumor individually or in smaller groups of tumors whose weight on the ninth day had been determined. On this day the increase in weight expressed in per cent. of the original weight during the period of treatment was ascertained. Before stating the effect of the various substances, it will be necessary to inquire into the degree of accuracy of our methods.

TABLE I.

Group No.	No. of tumors.	Calculated weight.	Actual weight.	Per cent.	
				+	−
1	21	6,525 gm.	5,717 gm. (+14%)	373%	331%
2	19	3,545 gm.	3,951 gm. (−11.4%)	17%	225%
3	10	2,550 gm.	2,575 gm. (> 1%)	26%	49%
4	15	2,910 gm.	3,377 gm. (14%)	52%	216%
		15,530 gm.	15,620 gm.	+468%	−821%
		−0.57%		−353%	

Average 5.8%

The first step in our second method consists in the calculation of the weight of the tumor on the ninth day on the basis of our drawings with the aid of weighed models. We investigated the accuracy of this determination by first calculating on several occasions the weight of a certain number of tumors nine days old by means of

drawings and then immediately removing the tumors and weighing them. We compared the weight of sixty-five tumors obtained first through calculation on the basis of drawings and models and subsequently through actual weighing. We arranged these into four groups according to the period in the course of our experiments at which the tumors were removed; in the first group are put together all those tumors removed in the beginning, at a time when the number of models at our disposal was still relatively small; in the fourth group those removed towards the latter part of our work, when the number of models had increased considerably.

In table I we find in the first column the weight calculated with the aid of drawings and models; in the second column the actual weight as determined with the aid of the balance. There is in the individual groups a maximum deviation of 14 per cent. in both directions, and if we add all the tumors there is found to be a little more than 0.5 per cent. error in the determination of weight by means of drawings and models. If we leave out of consideration the question of weight of the individual tumors and consider merely the means of the errors expressed in percentage of the actual weight, we find that on the average the weight of the individual tumors was underestimated. If we consider the fact that we examined usually two series of tumors at the same time and that therefore the error in both series which we wished to analyze was probably approximately of the same order and that the differences in weight which we produced experimentally were of a considerably higher order than the error of the method, and furthermore that all the experiments we made with this method were confirmatory of each other, we may conclude that the second method used is adequate for our purposes.

The reliability of the first method (palpation) we can test by determining in the same experiments the effect of the treatment by both methods. We first determine the increase in size by palpation and then the increase in weight by the second method. In weighing in a number of experiments each individual tumor, after its relative growth had previously been determined by palpation, and drawing and comparing its size on the thirteenth day with its original size on the ninth day, when the injections were started, we

could approximately come to a decision as to the accuracy of the first method.

TABLE II.

Comparison of Percentage of Growth of Tumors by Weighing and by Palpation.

	Tumors which by means of palpation had been diagnosed as retarded in growth.		Tumors which by means of palpation had been diagnosed as growing normally.	
	No. of mice.	Per cent. of growth (found through weighing).	No. of mice.	Per cent. of growth (found through weighing).
Series A II.				
Colloidal copper controls..	21	26%	14	70%
			In two cases we were uncertain, and both gave less than the average growth.	
Immunized mice.....	3	3%	17	102%
Series C II.				
Normal uninjected controls				
Class d.....	4	47%	11	203%
	In two cases we were uncertain whether retarded or not			
Class f.....	7	134%	28	294%
	In five cases we were uncertain whether retarded or not			
Mice injected with casein				
Class a.....	13	33%	10	97%
Class c.....	8	56%	8	163%
Class d.....	6	117%	4	236%
Class f.....	3	135%	4	291%
Mice injected with aurol				
Class a.....	12	68%	11	132%
Class c.....	4	12%	9	198%
Class d.....	3	−84%	8	202%
Class f.....	2	−54%	1	217%

Table II gives the results of these comparisons. We separated in each group those tumors which by palpation were found to show retarded growth from those showing normal growth, and determined in each the average increase in weight. The differences in actual weight between the two kinds of tumors are marked; and the results obtained by both methods agree, if we consider the various groups of tumors. In individual tumors there may occasionally be found a wrong diagnosis made by means of the first method.

In almost all cases the differences in actual weight between the tumors diagnosed as retarded in growth and growing normally are

more than 100 per cent. We have to consider that we must in each case compare tumors of the same class, inasmuch as tumors grow, as a rule, more rapidly from the ninth to the thirteenth day, the smaller they were on the ninth day; therefore, the tumors of class f grow more rapidly than tumors of class d; tumors of class d grow more rapidly than tumors of class c, and tumors of class c grow more rapidly than tumors of class a; and that therefore in class f a relative increase is regarded as retarded growth which in class a would have been considered as marked growth.

In other experiments we did not weigh each individual tumor, but certain groups of tumors, those tumors being in each case grouped together which had a similar weight on the ninth day. In this case we could less definitely determine how far the results obtained by both methods agreed, but, as far as could be judged, the results agreed fairly well.

In order to eliminate as much as possible the subjective factor in the first method, two persons made in a certain number of cases parallel experiments, using palpation and drawing as a means of determining the growth of the tumors; both persons arrived independently at the same conclusions. Furthermore, as far as the principal problems are concerned, we used both methods and the results obtained by both methods were on the whole confirmatory of each other. We may therefore conclude that the first method is sufficiently accurate to permit us to determine in a general, but not in an exact, quantitative way, whether or not a certain experimental interference had a retarding influence on the tumor growth. In a number of our earlier experiments we used, however, the first method exclusively, and we shall distinguish between results obtained by both methods and those obtained only by the first method.

We examined by these methods and within the time interval stated the action of various classes of substances, distilled water, a number of inorganic salts, a number of inorganic colloidal substances, various organic colloidal and non-colloidal substances, and especially hirudin alone and in combination with some other substances. We limited the treatment in the large majority of cases to four intravenous injections given on four consecutive days, because it was necessary to treat all animals in a comparable way. In ex-

periments on which we shall report in a further communication the four intravenous injections were preceded by a preliminary set of four intravenous injections. It is often difficult to give more than eight intravenous injections to a mouse, and we used, therefore, four injections as the standard for comparison.

In tables III, IV, V, and VI, those of our experiments in which the first method was used are grouped together. We determined first the lethal dose of each substance to be used. The quantity given varied somewhat in the case of the different substances. It was sometimes half the lethal dose, occasionally less, and in the majority of the cases somewhat more than one half the lethal dose. In the second column of our tables the lethal doses are represented, in the third column the dose given in our experiments. The fourth column gives the coefficient of safety; namely, the relation between the lethal and experimental dose. We used two criteria in judging the action of the various substances.

(1) One day after the first injection we examined the tumors, making incisions into them. Some substances exerted a distinct effect on the tumor after one injection. In columns 5 to 9 we gave the result in cases in which these examinations were made. Such examinations one day after the injection were not made in all the substances. We called "much affected" tumors those in which on the tenth day a large part of the growth was found to be necrotic and softened and sometimes hemorrhagic in the central portion. Some living tumor tissue may still be found in the periphery. In tumors which are designated as "partly affected" there is still a slight softening in the center of the tumor, but at least one half of the peripheral portion is not affected. In tumors "slightly affected" there is a very small area of softening in the central portion of the tumor, but most of the tumor appears normal.

(2) In columns 10, 11, and 12, we give the result of the effect of four injections on tumor growth, the figures showing the number of tumor mice observed. "Retarded growth" shows those tumors which grew distinctly less than the average tumor of a certain size. A certain number of tumors became distinctly smaller under the influence of the treatment. They are marked "retrogressing." It happens, of course, occasionally that a tumor retrogresses spon-

taneously, especially a tumor of small size; but this is on the whole not a usual occurrence. Only those mice were considered which lived through the whole period of the experiment, while those, for instance, which died on the fourth day were not included.

A number of the substances were obtained from manufacturers; others were prepared in our laboratory by Professor E. H. Keiser. Colloidal copper and platinum were prepared in accordance with Bredig's method.[2] Various organic substances were prepared according to the ordinary methods of biological chemistry.[3]

If we now consider the results in table III, we find that intravenous injection of distilled water did not retard the growth of tumors, neither did one injection cause any noticeable changes in the tumors. Two sulphur preparations (milk of sulphur, and hydrochloric acid plus sodium thiosulphate) were examined after one injection. No effect was noted. Various inorganic salts (copper nitrate, copper ammonium sulphate, mercury bichloride, gold sodium chloride (Merck), sodium tartrate, lanthanum nitrate) did not show any

[2] Some other substances were prepared in the following manner:

Copper Casein B.—Add a concentrated solution of $CuSO_4$ to an equal amount of a saturated solution of casein (dissolved in NaOH). Dissolve the precipitate in NH_4OH and precipitate with absolute alcohol.

Copper Casein C.—Dissolve copper tartrate in NaOH and add to an equal quantity of saturated casein solution (dissolved in NaOH). Precipitate in absolute alcohol.

$H_2S + NaNO_2$.—Pass H_2S through a moderately strong $NaNO_2$ solution; this gives a yellow precipitate. Dilute with H_2O. After standing for twenty-four hours the precipitate is dissolved.

Sulphur.—Colloidal (chemically prepared). Heat flowers of sulphur with NaOH and a little water; boil and pass steam through a condenser. This gives a milky liquid with slight reddish color.

Selenium.—Colloidal (chemically prepared). Dissolve the selenium by boiling with concentrated HNO_3, and evaporate this to dryness. Heat and sublime the SeO_2. Dissolve the crystals in H_2O and then run in H_2S. This gives an orange colored liquid.

Casein and Sulphur.—Dissolve 10 gm. of casein (purified) in 600 c.c. of distilled water to which 20 c.c. of normal NaOH have been added. Pass a stream of H_2S through this for from twelve to twenty-four hours, until saturated with H_2S. This gives an acid reaction to litmus. Then draw air through until the H_2S is no longer perceptible.

[3] Protamin was prepared according to Kossel's method from dried salmon milt, which we obtained through the kindness of Professor Alonzo E. Taylor, of the University of Pennsylvania.

TABLE III.

Substance.	Lethal dose.	Usual dose.	Coefficient of safety. Lethal dose: usual dose.	One injection.				Several injections.		
				Much affected.	Partly affected.	Slightly affected.	Normal.	Retarded growth.	Retrogressing.	Normal growth.
Copper nitrate m/250	0.6 c.c.	0.4 c.c.	1 : 0.66	—	—	—	—	2	—	25
Copper ammonium sulphate 0.7%	—	0.3 c.c.	—	—	—	—	—	1	—	10
Mercury bichloride m/400	0.4 c.c.	0.21 mg. 0.25 c.c.	1 : 0.62	—	—	—	—	—	—	5
Lanthanum nitrate	4.0 mg.	2.5 mg.	1 : 0.62	—	—	—	—	—	—	3
Sodium tartrate 3⅛%	0.5 c.c.	0.4 c.c.								
	17.0 mg.	14.0 mg.	1 : 0.8	—	—	—	—	—	—	15
Gold potassium cyanide (Merck)	0.15 mg.	0.05 mg.	1 : 0.33	—	—	—	—	5	—	17
Gold sodium chloride (Merck)	0.8 mg.	0.4 mg.	1 : 0.5	—	—	—	—	1	—	20
Distilled water	—	1.0 c.c.	—	—	—	—	3	2	—	19
Milk of sulphur	0.75 c.c.	0.37 mg. 0.16 c.c.	1 : 0.5–0.25	—	—	—	3	—	—	—
Hydrochloric acid + sodium thiosulphate	0.75 c.c.	0.5 mg. 0.25 c.c.	1 : 0.66–0.33	—	—	—	7	—	—	—

TABLE IV.

Substance.	Lethal dose.	Usual dose.	Coefficient of safety. Lethal dose: usual dose.	One injection.				Several injections.		
				Much affected. (1)	Partly affected. (2)	Slightly affected. (3)	Normal.	Retarded growth.	Retrogressing.	Normal growth.
Colloidal copper	2.5 c.c. +	1 c.c.	1 : 0.4–	—	—	1	18	371	—	277
Colloidal copper hydrate (Mulford)	0.1 c.c.	0.05 c.c.	1 : 0.5	—	—	—	—	2	1	20
Colloidal copper (Heyden) 0.3%	0.7–0.4 c.c.	0.25 c.c.								
	2.1–1.2 mg.	0.75 mg.	1 : 0.35–0.62	15	—	1	2	10	—	4
Colloidal platinum	2.5 c.c. +	1.0 c.c.	1 : 0.4–	—	—	—	—	12	—	3
Colloidal sulphur (electrolytic)	—	1.0 c.c.	—	—	—	—	—	3	—	7
Colloidal sulphur (chemical)	—	1.0 c.c.	—	—	—	—	—	1	—	10
Colloidal selenium (Mulford)	0.1 c.c.									
	0.025 c.c.	0.0125 c.c.	1 : 0.125–0.5	—	—	—	—	6	—	15
Colloidal selenium (prepared by us chemically)	—	1.0 c.c.	—	—	—	—	—	—	—	11
Aurol (Hille) 15%	0.7 c.c.	0.5 c.c.	1 : 0.72	—	—	—	—	14	7	29
Copper casein A	1.2 mg.	1.0 mg.	1 : 0.83	—	—	—	—	8	—	3
Copper casein B	1.2 mg.	1.0 mg.	1 : 0.83	—	—	—	—	—	—	9
Copper casein C	12 mg.	10.0 mg.	1 : 0.83	—	—	—	—	11	—	—

noticeable inhibiting effect on tumor growth. Although the number of tumor mice treated was not great in the case of each individual substance, the number is sufficiently large (eighty-three) if we add animals used in the various experiments. We may therefore conclude that inorganic salts injected under the conditions of our experiments had not a sufficiently marked retarding influence on tumor growth to be accessible to determination by our first method. This does not exclude the possibility that through our second method a slight influence might be found. Only in the case of gold potassium cyanide was there possibly a slightly retarding influence present, but if present at all, it certainly was not marked.

In table IV some inorganic colloidal substances and combinations between copper and casein are considered. Besides the colloidal copper prepared by us electrolytically in accordance with Bredig's method we used a preparation of colloidal copper made by the H. K. Mulford Company, and one by Heyden (Dresden). The exact composition of these two preparations is not known to us. Colloidal platinum we prepared ourselves in accordance with Bredig's method. Colloidal sulphur we prepared either chemically or by the electrolytic method of Svedberg. Aurol (Hille) is a preparation of colloidal gold which was sent us by the Vitochemical Laboratories, Chicago.[4] Professor Keiser prepared several combinations of copper and casein (B and C).

The results are as follows: Colloidal copper prepared electrolytically, colloidal copper (Heyden), colloidal platinum, aurol (Hille), and two of the copper casein preparations had a distinct retarding influence on tumor growth; while the colloidal copper hydrate (Mulford), colloidal sulphur prepared chemically, colloidal selenium prepared by us chemically, and one copper casein preparation (B) were without decided influence. It is possible that the copper casein preparation B had undergone secondary changes at the time we used it and that this fact may account for the negative results. In the case of the electrolytically prepared sulphur and colloidal selenium (Mulford) there was perhaps a slight effect present, but it was certainly not marked. It is possible that small quantities of colloidal

[4] The H. K. Mulford Co. and the Vitochemical Laboratories kindly put their preparations at our disposal for experimental purposes.

platinum were admixed to the solution of electrolytically prepared sulphur and that this may account for a slight effect which was possibly present.

Our method does not permit us to differentiate quantitatively between the efficacy of the various active substances. We observed a temporary retrogression during the period of injection in a few animals treated with aurol; but the number of retrogressing tumors in this case is too small to be of much significance. On the whole, however, the difference in the efficacy of the various preparations does not seem to be striking. Two preparations were tested as to their early effect on the tumor after one injection. Our own preparation of colloidal copper was without effect in this respect, while the Heyden preparation of colloidal copper had a marked effect.

We may then conclude that in contradistinction to the inorganic salts of molecular dispers character, which are without marked effect, certain colloidal solutions of heavy metals (copper, platinum, gold) are effective, inasmuch as they retard definitely the growth of a number of tumors of injected animals; of a similar activity are also combinations of copper salts and casein. However, according to the method of preparation of some of these substances their efficacy differs. None of these substances has a markedly destructive action on mouse tumors. In the majority of cases the effect is transitory and does not lead to a cure.

In table V the results of the injections of various organic substances are represented. We used various albuminous substances, as well as starch and lecithin, as representative of the carbohydrates and lipoids. In addition we used horse serum; tuberculin we used as a representative of bacterial toxins. Ethylhydrocuprein was successfully used by Morgenroth[5] in the treatment of pneumococcus infections. Ergamin is a substance producing symptoms somewhat resembling anaphylactic shock. Of all these substances only casein and nucleoproteid preparations had a sufficiently retarding effect on tumor growth to be demonstrable by our method. And the effects of these substances seemed to be on the whole of a similar order to those produced by the colloidal metals. They differ, however, from

[5] We are indebted to Professor Morgenroth and the manufacturers for the material at our disposal.

TABLE V.

Substance.	Lethal dose.	Usual dose.	Coefficient of safety. Lethal dose: usual dose.	One injection.				Several injections.		
				Much affected.	Partly affected.	Slightly affected.	Normal.	Retarded growth.	Retrogression.	Normal growth.
Ethylhydrocuprein 0.375% ...	0.4 c.c.	0.25 c.c.								
	1.5 mg.	0.94 mg.	1 : 0.62	—	—	—	—	—	—	4
Ergamin (Burroughs and Wellcome).....	—	1.0 mg.	—	—	—	—	—	—	—	6
Starch 5% suspension.....	1.0 c.c. +	0.75 c.c.	1 : 0.75 +	—	—	—	11	—	—	6
Lecithin 0.1% (Merck ovo)...	0.75 c.c.	0.5 c.c.								
	0.75 mg.	0.5 mg.	1 : 0.66	—	—	—	6	—	—	6
Pepton 5%	1.5–1.25 c.c.	0.6 c.c.								
	0.1–0.075 mg.	0.03 mg.	1 : 0.4–0.5	—	—	—	13	101	—	21
Protamin 0.1% .	0.7 c.c.	0.5 c.c.								
	0.7 mg.	0.5 mg.	1 : 0.72	—	—	—	10	—	—	10
Serum globulin 15%.....	2.0–1.5 c.c.	1.0 c.c.								
	0.3–0.22 gm.	0.15 mg.	1 : 0.5	—	—	—	23	—	—	9
Ovalbumin saturated	1.5–1.0 c.c.	0.75 c.c.								
	0.37–0.25 gm.	0.18 gm.	1 : 0.5–0.75	—	—	—	102			23
Tuberculin (AT) 50%.....	0.65–0.2 c.c.	0.5–0.15 c.c.	1 : 0.8–0.75	—	—	—	14	—	—	9
Horse serum.....	1.5 c.c.	1.0 c.c.	1 : 0.66	—	—	—	20	—	—	4
Casein 1⅔%.....	2.0 c.c.	1.0 c.c.								
	32.0 mg.	16.0 mg.	1 : 0.5	46	6	20	22	68	—	54
Casein 1⅔% + sulphur.....	2.0 c.c.	1.0 c.c.								
	32.0 mg.	16.0 mg.	1 : 0.5	66	15	23	3	34	—	16
Nucleoproteid (lymph gland of cattle) 15%....	0.75–0.25 c.c.	0.5–0.15 c.c.								
	113.0–38.0 mg.	76.0–22.0 mg.	1 : 0.66	18	1	4	28	7	—	5
Nucleoproteid (kidney of cattle) 15%.....	0.25–0.15 c.c.	0.2–0.1 c.c.								
	38.0–22.0 mg.	30.0–15.0 mg.	1 : 0.8–0.66	10	2	5	13	17		9

the electrolytically prepared colloidal copper through the edema or hemorrhages which they cause and which is noticeable twenty-four hours after the first injection in many tumors of the injected mice. Here again casein and nucleoproteid were the only active substances, while all the other substances in table V were without direct effect on the tumors.

In table VI the effect of hirudin and various combinations of hirudin and other substances is given. On the whole, hirudin is, as far as the number of affected tumors is concerned, not more effective than colloidal copper. It differs, however, from colloidal copper in

TABLE VI.

Substance.	Lethal dose.	Usual dose.	Coefficient of safety. Lethal dose: usual dose.	One injection.				Several injections.		
				Much affected.	Partly affected.	Slightly affected.	Normal.	Retarded growth.	Retrogression.	Normal growth.
Hirudin 0.25% solution	1.5–0.1 c.c.	0.05–1.0 c.c.	1 : 0.5–0.065	11	—	—	—	114	70	102
	3.7–0.25 mg.	2.5–0.125 mg.								
Hirudin + nucleoproteid	—	Usual dose of hirudin + usual dose of nucleoproteid		10	—	—	—	1	11	2
Colloidal copper + hirudin	—	Colloidal copper 0.5–0.25 c.c. Hirudin 0.125–0.25 mg.		—	—	—	—	11	24	6
Different one each day: colloidal copper, casein, hirudin, nucleoproteid	—	Usual doses		—	—	—	—	34	—	12
Colloidal copper, intravenously. Hirudin, subcutaneously	—	Colloidal copper 1.0 c.c. Hirudin 0.80 mg.		—	—	—	—	7	—	6
Hirudin, subcutaneously 0.25%		0.25 c.c. 0.8 mg.		—	—	—	—	2	—	9

two respects: (1) the number of tumors retrogressing under the influence of hirudin is considerably greater; and (2) one single injection has a marked effect on the tumor, causing hemorrhage and edema. As we saw previously, colloidal copper did not have such an effect. The retrogression of the tumors is usually not a permanent one; but after temporary cessation of the injections the tumors begin in the majority of cases sooner or later to grow again; a certain number of the tumors, however, retrogress definitely. Hirudin

given subcutaneously was without a marked effect. It is, however, to be noted that the efficacy of several samples of hirudin differed and that the results obtained at different periods were not equally marked. More effective than hirudin alone was a combination of hirudin and either nucleoproteid or colloidal copper, both substances being given at the same time. In combination with nucleoproteid the number of retrogressions was considerably greater than in cases in which hirudin alone was used. It is, however, to be considered that at that time the hirudin which we used was stronger than some later samples that we received. A combination of colloidal copper and hirudin was also effective. The number of retrogressing tumors was here very great, much greater than with either hirudin or colloidal copper alone. This combination is also highly toxic and a large number of the tumor mice injected died. If the hirudin was given subcutaneously instead of intravenously, while the colloidal copper was given intravenously, the effect was merely that of the colloidal copper. Hirudin administered subcutaneously was therefore again ineffective.

As we shall see later, processes of immunization take place in the course of a series of injections. It was therefore of interest to test the effect of a combination of four different substances, a new substance being given on each successive day. Colloidal copper, casein, hirudin, and nucleoproteid were thus alternately administered. There was, perhaps, a somewhat stronger effect than in cases in which each of these substances was given alone for four successive days, but the difference was not marked. This result points to the conclusion that one single injection of a certain substance does not lead to the production of a noticeable degree of immunity.

In addition to these experiments carried out by means of the first method, we carried out a series of experiments with the second method, in order to obtain results of a more quantitative character.

All the tumors treated in an experiment in the same manner represented a series. Inasmuch as the percentage increase of tumors in weight or volume depends on their weight at the time when the experiment was started, we had to subdivide a series in different classes, according to the weight of the tumors on the ninth day. In

all experiments the classes were as follows: class a = tumors weighing 360 milligrams or more; class c = tumors weighing between 230 and 360 milligrams; class d = tumors weighing between 130 and 230 milligrams; class f = tumors weighing between 25 and 130 milligrams.

In cases in which we were unable to weigh each tumor at the end of the experiment, we divided the various classes into smaller groups, according to the weight of the tumors on the ninth day, and all the tumors of one group were weighed together. In table VII the results obtained by the second method are given.

In the first column we find the number of mice used in testing each substance. In the case of pepton and ovalbumin we referred separately to the individual experiments made with each of these substances. In addition we give a summary of the three experiments done with each of these two substances. In the third experiment we give two sets of figures; in one set all the tumors were included; in the corrected set we omitted from consideration the various retrogressing tumors. This seemed to be a fairer way of calculation, because in this experiment there were also a number of retrogressions in the control tumors. In all our tables only the tumors of such animals were included as lived to the end of the experiment. All animals that died before that time were omitted. The last four columns of the table (a, c, d, f) give the percentage increase in weight in the different classes of tumors arranged in accordance with their weight nine days after inoculation; a represents the largest, f the smallest tumors. Columns 2, 3, 4, and 5 (α, β, γ, δ) give the average percentage increase of the tumors of a series. We describe in the following paper[6] on "Immunization against substances inhibiting tumor growth" the methods used by us for the determination of these averages.

In the first line in table VII the percentage increase of normal control mice is given. The second line gives the corresponding figures for mice injected with colloidal copper. We recognize clearly the depressing effect of colloidal copper on tumor growth. While the normal control tumors gain 170 to 180 per cent. in weight in four days, in the tumors of the injected animals, including those in

[6] Fleisher, M. S., Vera, M., and Loeb, L., *Jour. Exper. Med.*, 1914, xx, 522.

TABLE VII.

	No. of mice.	α	β	γ	δ	a	c	d	f
Normal controls	262	177%	192%	174%	180%	117%	140%	181%	251%
Colloidal copper	300	103%	107%	110%	107%	81%	81%	123%	155%
Aurol	50	111%	112%	97%	106%	[23]98%[7]	[13]137%	[11]141%	[8]11%
Hirudin	69	139%	150%	147%	145%	——	[7]126%	[27]150%	[35]166%
Casein	56	84%	112%	138%	111%	[23]61%	[15]104%	[10]158%	[7]228%
Pepton 1	42	111%	154%	131%	121%	[3]60%	[5]145%	[13]132%	[21]185%
Pepton 2	52	191%	192%	183%	189%	[3]135%	[4]195%	[17]220%	[28]181%
Pepton 3	28							[14]157%	[14]108%
Corrected, omitting retrogressing tumors *similar to those in controls*	22							[12]177%	[10]213%
Pepton. Summary	122	152%	173%	157%	155%	94%	170%	169%	158%
Pepton. Summary. Corrected figures.	116							176%	193%
Ovalbumen 1	39	185%	174%	176%	177%	[1]120%	[2]236%	[14]182%	[22]166%
Ovalbumen 2	48	166%	192%	159%	172%	[4]99%	[6]123%	[15]182%	[23]233%
Ovalbumen 3	53	145%	161%	157%	154%	[8]99%	[14]134%	[14]118%	[17]277%
Corrected, omitting retrogressing tumors	46	160%	205%	191%	185%	[7]120%	[13]145%	[12]176%	[14]325%
Ovalbumen. Summary	140	165%	176%	164%	168%	106%	164%	161%	225%
Ovalbumen. Summary. Corrected figures	133	170%	191%	175%	178%	113%	169%	180%	241%

[7] The small figures at the upper left side of some of the percentage figures in the last four columns indicate the number of mice used in the various experiments.

which no effect was noticeable, as well as those which were influenced by the colloidal copper, the increase was from 100 to 110 per cent.

As will be seen from the tables, our figures in the case of colloidal copper are based on the examination of a large number of mice.

Aurol is effective, but probably less so than colloidal copper. In this experiment some of the smaller tumors retrogressed and the figure for f is therefore very low. The low percentage increase in group f reduces considerably the average increase of the tumors treated with aurol. Inasmuch as the number of mice in class f was very small, we must not attach too much importance to this figure. In the other classes the reduction in percentage increase in weight was considerably less than in colloidal copper. In the case of aurol we noticed a dark bluish grey discoloration of the tumors, liver, and occasionally the spleen, after four injections had been given. Apparently this discoloration was due to a deposit of foreign material in capsule and stroma.[8]

Hirudin also had a distinctly inhibiting effect. The full effect can, however, not be appreciated from a study of our figures, as there was much coagulated blood and marked edema in the tumors of the injected mice. The effect of hirudin is therefore more marked than is indicated by the figures of our table. Besides, a number of these tumors are usually found to retrogress when the observation of the tumors of mice injected with hirudin is prolonged.

Casein is also effective, but perhaps not quite so effective as colloidal copper. Here the average increase determined according to the methods is especially low because the number of the large tumors was great and their growth was unusually retarded.

While casein served as a representative of the albuminous substances which we had found effective in employing the first method of measurement, we used pepton and ovalbumin as representatives of those proteids which the first method showed to be ineffective, or less effective. With each of these two substances we made three experiments. In the first experiment the mortality among the in-

[8] We intend to examine the character of the deposit more closely as soon as possible. On fixed and stained specimens the cause of the discoloration could not be determined.

jected mice had been very great, about one half of all the injected mice dying after the first injection. The remaining mice also were somewhat affected. The mice injected with pepton showed very much retarded tumor growth. The effect was here similar to colloidal copper. The mice injected with ovalbumin behaved differently in different classes. In this experiment the first dose of the substances given had evidently been too strong and the general health of the mice may have suffered in consequence. We may state that neither after injection of colloidal copper nor of casein does the general condition of the mice seem to suffer.

In the second experiment an effect on the part of pepton as well as ovalbumin was either absent or very small. A slight reduction in class f must not be overestimated, inasmuch as in class f spontaneous retrogressions are apt to occur. In the third experiment there occurred in the control experiments a number of spontaneous retrogressions of tumors. Retrogression of tumors in the animals injected with pepton or ovalbumin must therefore in the main not be attributed to the action of these substances, and the mice with retrogressing tumors should be entirely omitted, in order to obtain a fair estimate of the action of these substances. With these corrected figures ovalbumin was without effect in the third experiment, while the original figures indicated some effect of ovalbumin, which was, however, considerably less than casein. With pepton only tumors of classes d and f were used in this experiment. There was in this case some effect in class f noticeable in using either of the two figures. The corrected figures of class d were approximately the figures of normal mice, while the uncorrected figures were similar to those of casein. If we consider the summary of the three experiments we find that pepton has decidedly less effect on the tumors than casein, although it has some effect, while ovalbumin is almost without effect. These experiments make it probable, therefore, that ovalbumin and pepton are decidedly less effective than casein, although they may have some effect, especially pepton; and they confirm therefore, on the whole, the conclusions based on the first method. We believe, however, that further experiments are necessary to make our conclusion concerning the different efficacy of various proteids definite.

While in the case of most substances which we used we studied the effect on the growth between the ninth and thirteenth day after the inoculation of the tumor, we determined in the case of colloidal copper and of hirudin in addition the effect of injections given from the second to the sixth day after inoculation. We found that the tumors of animals treated in this way were on the ninth day after inoculation as large as tumors of animals which had not been injected. It seems, therefore, that injections given at an early date are without a noticeable effect on tumor growth. This is perhaps due to the fact that at the early period the vascularization of the tumor has not yet progressed as far as later, and perhaps also to the lesser interference with the circulation through pressure at this early period. If, however, the tumors have reached a certain development, such as is found between the ninth and twelfth days after inoculation, they are much more accessible to the action of colloidal copper.

TABLE VIII.

Decrease of Percentage Increase in Weight under the Influence of Colloidal Copper in the Different Classes.

Group.	Tumors. Control mice.	Increase in weight of tumors in mice injected with copper.
a	100 %	69 %
c	100 %	58 %
d	100 %	68 %
f	100 %	62 %

It was of interest to determine whether there was any difference between small and large tumors in the degree with which they are influenced by the injection of colloidal copper. In table VIII the tumors are arranged in classes, a, c, d, f, according to their size. If we take 100 per cent. as the standard of the average increase in weight of the various classes of control tumors, the tumors in the various classes of injected mice lose approximately in the same proportion under the influence of colloidal copper. Tumors of different size are therefore relatively influenced in a similar manner through the injections of colloidal copper. While the relative loss in all classes is approximately the same, the absolute loss is of course greater, the greater the percentage increase in weight of the different classes in normal control mice.

SUMMARY.

We have described the methods used in determining the influence of certain substances on tumor growth, and we measured approximately the degree of reliability of the quantitative method used. We examined with these methods various classes of substances,—distilled water, a number of inorganic salts, inorganic colloidal substances, various organic colloidal and non-colloidal substances, especially various proteids, tuberculin and hirudin alone as well as in combination with other substances. Distilled water, various inorganic sulphur preparations, and various inorganic salts did not show an inhibiting effect on tumor growth sufficient to be detected by means of our first method. Only in the case of gold potassium cyanide was there possibly a slightly retarding influence present. On the other hand, certain colloidal solutions of heavy metals (copper, platinum, gold) retard the growth of a number of tumors of injected animals. Certain combinations of copper salts and casein act in a similar manner. Of the organic substances used, casein, nucleoproteid, and hirudin were active, while the other proteids tested, as well as various other organic substances and tuberculin and lecithin, seemed to be either without effect or weaker than the other substances mentioned as retarding the tumor growth. Hirudin was active and caused in addition to its inhibiting influence the retrogression of a certain number of tumors. Especially active was a combination of hirudin with colloidal copper and of hirudin with nucleoproteid.

One single injection of casein or nucleoproteid, or of the Heyden preparation of colloidal copper, leads to a more or less marked edematous condition of a certain number of tumors, while hirudin caused in addition, in many cases, marked hemorrhages in or around the tumors. Other substances which we tested did not show this effect, although their inhibiting action on tumor growth may have been equally strong. Very young tumors (two to six days old) are not retarded in their growth through injection of colloidal copper or hirudin, while nine to thirteen days old tumors are, independently of their size on the ninth day, inhibited in approximately the same relative degree; absolutely, however, the more rapidly growing smaller tumors are more markedly inhibited than the normally more slowly growing larger tumors.