PROPERTIES OF THE CAUSATIVE AGENT OF A CHICKEN TUMOR

XIV. Relation between a Tumor Nucleoprotein and the Active Principle

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Repeated attempts have been made in the past to correlate the tumor-producing activity with the chemical constitution of chicken tumor extracts, and the ultraviolet absorption spectrum of these extracts has been used for that purpose on several occasions. Ordinary Berkefeld filtrates of Chicken Tumor I were found to possess a marked absorption in the ultraviolet region, at $\simeq \lambda 2600$, and a weaker range of absorption at $\lambda 4100$ (1). Extracts purified by adsorption and dialysis retained their tumor-producing property, and still absorbed ultraviolet light, with the suggestion of a maximum at $\simeq \lambda 2550$ (1). Recently, a fraction which carried practically all the initial tumor-producing activity was separated from chicken tumor extracts by means of differential centrifugation at high speed. The material, purified in this manner, was found to exhibit an ultraviolet absorption spectrum strikingly similar to that of the tumor extract purified by adsorption and dialysis, with indication of a maximum of absorption at $\lambda 2575$ (2). The fact that active tumor fractions, although prepared by entirely different methods, possess the same absorbing power for ultraviolet light led us to reinvestigate the relation between the absorbing elements and the tumor principle.

Materials and Methods

Preparation of Tumor Extracts.—The mode of preparation of purified and highly active tumor material has been briefly described in previous notes (3).

The tissue from selected tumors, frozen at −80°C. and stored at that temperature for 3 to 15 days, was used in these experiments. The frozen tissue was ground with sterile sand and extracted with a 0.005 M phosphate buffer solution at pH 7.0. This tissue suspension was centrifuged for 30 minutes at 2400 times gravity, in an angle

centrifuge.[1] The deposited tissue was extracted once more in exactly the same manner and the two supernatant fluids were combined. This material will be referred to as the tumor extract. The total volume of buffer solution used for the double extraction corresponded to 15 times the weight of the tumor tissue. The average solid material of this tumor extract was about 3.4 mg. per cc., and the specific viscosity of the fluid was approximately 3.2 times that of water.

Purification of the Active Fraction in the High Speed Centrifuge.—The tumor extract was centrifuged under a force of 17,000 to 18,000 times that of gravity for a period of 2 to 3 hours, depending on the viscosity of the fluids.[2] The supernatant fluid was discarded and the sediment was resuspended in a small volume of phosphate buffer solution. Coarse particles were removed from the suspension by a short run of 3 minutes at high speed. The coarse sediment was resuspended in buffer solution, redeposited by a short run of 3 minutes, and discarded. The supernatant fluids from the different short runs, which were found to contain the active principle, were combined and saved for further purification in the centrifuge.

The entire process, consisting in a long run followed by two or three successive short runs of 3 minutes at high speed, was repeated twice more. In the end, the purified tumor agent was taken up in 0.005 M phosphate buffer solution at pH 7.0, the final volume being equal to one-tenth that of the original extract. This last suspension will be referred to as the "purified fraction." During the experiment, the temperature of the material was maintained near 0°C., except for the first long run at high speed, when it attained 12–16°C.

Tests of Activity.—Chicken tumor extracts gradually lose their activity *in vitro* and the tumor agent appears to be even less stable in the purified form. For this reason, efforts were made to test the purified fraction the day of its preparation, extraction of the frozen tissue and treatment of the extract in the high speed centrifuge requiring 12 to 14 hours.

The tumor-producing power of the purified material was determined by injecting 0.4 cc. of the test solutions into the skin of adult Plymouth Rock hens. Dilutions of the stock solutions were made with a 0.005 M phosphate buffer solution at pH 7.0, containing 2 per cent rabbit serum. New 1 cc. pipettes were used for the first three dilutions. The results were recorded by measuring the size of the tumors which were present, not later than 18 days after inoculation.

Determination of Ultraviolet Absorbing Power.—Measurements of the absorbing power of the solution for ultraviolet light were carried out with a Hilger sector-photometer as previously described (1). In recording the curve, the extinction coefficients were calculated from the relation $I = I \times 10^{-\epsilon c}$ where c was the concentration arbitrarily expressed in grams per 10 cc. In a few experiments, the source of light was the continuous spectrum of a hydrogen discharge tube. Absorption measurements were carried on the freshly prepared material at the same time at which inoculation tests were performed.

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[1] International Equipment Co., Boston, type S.B., size 1 centrifuge in conjunction with conical head No. 283.

[2] The device used in these experiments was the multispeed attachment and No. 295 head provided by the International Equipment Co., Boston, for their type S.B., size 1 centrifuge.

EXPERIMENTAL

Tumor-Producing Activity of the Purified Tumor Fraction and Ultraviolet Absorption Spectrum

The results obtained in the following experiment are typical for the purified fraction prepared according to the method described above.

Tumor-Producing Power of the Purified Fraction.—The results of inoculation, based on four tests at each dilution, are given in Table I. It shows that the purified fraction was fully active when brought to a dilution of 6.5×10^{-10}, as calculated from the weight of the original tumor tissue. Inoculation of 4.0×10^{-13} gm. of the purified substance was sufficient to produce well developed tumors 18 days after injection. In this experiment,

TABLE I

Tumor-Producing Power of the Purified Fraction

Results of Activity Tests 18 Days after Injection

Dilution	Tumor extract (control)			Purified fraction		
	Solids injected	Takes	Tumor size	Solids injected	Takes	Tumor size
	gm.	*per cent*	*cm.*	*gm.*	*per cent*	*cm.*
6.5×10^{-5}	1.7×10^{-5}	100	2.3 × 1.8	4.0×10^{-8}	100	2.5 × 1.9
6.5×10^{-6}	1.7×10^{-6}	100	2.5 × 1.6	4.0×10^{-9}	100	1.8 × 1.6
6.5×10^{-7}	1.7×10^{-7}	100	1.9 × 1.5	4.0×10^{-10}	100	1.9 × 1.6
6.5×10^{-8}	1.7×10^{-8}	75	1.5 × 1.2	4.0×10^{-11}	100	2.1 × 1.8
6.5×10^{-9}	1.7×10^{-9}	100	1.4 × 1.1	4.0×10^{-12}	100	1.8 × 1.4
6.5×10^{-10}	1.7×10^{-10}	0	—	4.0×10^{-13}	75	1.5 × 1.2

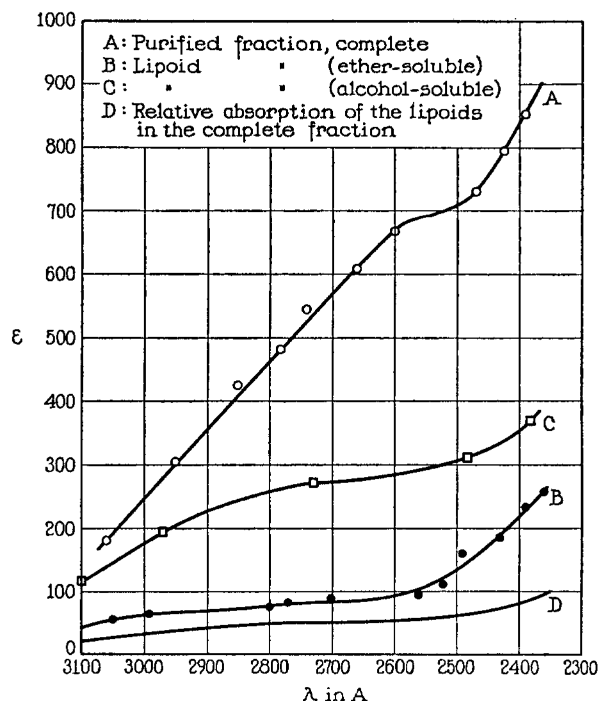
as can be seen from Table I, the purified fraction exhibited a tumor-producing power at least ten times greater than that of the unfractionated extract, kept as control at 4°C. This relative enhancement of activity shown by the material purified by centrifugation was observed consistently.

Absorption Spectrum of the Purified Fraction.—The ultraviolet absorption spectrum of the above material was determined. The preparation was a 0.017 per cent solution of the purified substance in 0.005 M phosphate buffer at pH 7.2.[3] As shown by the results of activity tests, this solution contained at least 4.2×10^{8} active doses per cc. Its ultraviolet absorption spectrum is given in Text-fig. 1, curve A, which shows a maximum at λ2575. This curve is quite similar to that obtained for nucleoproteins (4).

When the present results are compared with previous data (1, 2) it is

[3] The hydrogen ion concentration of all solutions studied was determined by means of a glass electrode.

apparent that improved methods of purification are reflected in a marked increase in the ultraviolet absorbing power of the purified fraction, and this is paralleled by an increase in tumor-producing activity. At λ2575, the extinction coefficient of the fraction purified by adsorption and dialysis and subsequently concentrated, was $\epsilon = 135$ (1). Material purified by high speed centrifugation, but with no more than 10 to 20 per cent of the



TEXT-FIG. 1. Absorption spectra of the purified tumor fraction and of its lipoid constituents.

activity of the original extract, showed, for the same wave length, an extinction coefficient $\epsilon = 525$ (2). In the present work the extinction coefficient of highly active preparation was found to be consistently in the neighborhood of $\epsilon = 700$ for the wave length λ2575. In each case, however, the absorption curve is essentially the same, with the maximum at practically the same wave length, λ2575. From these observations, it seems that a correlation may exist between the absorbing power and the activity of the solution.

Absorbing Power of the Lipoid Constituents of the Purified Fraction.—Previous studies have shown that part of the purified fraction was lipoid in

nature, the other portion being represented, to a large extent, by a nucleoprotein (5).

Separation of the lipoids from the purified fraction was accomplished by extracting the dried substance with ether and alcohol. The freshly purified tumor fraction was frozen at −80°C. and desiccated *in vacuo* in the frozen state. Extraction was performed at room temperature by leaving the dry substance in contact with the solvent for successive periods of 2 hours, with occasional stirring. The material was extracted three times with ether, and then three times with absolute alcohol. In terms of dry weight, the ether-soluble and the alcohol-soluble portions represented 15.4 and 14.3 per cent of the original material, respectively. No purification of these lipoids was attempted, each fraction being probably a mixture of several components. For the spectrographic measurements, the ether and alcohol fractions were dissolved in heptane and examined separately.

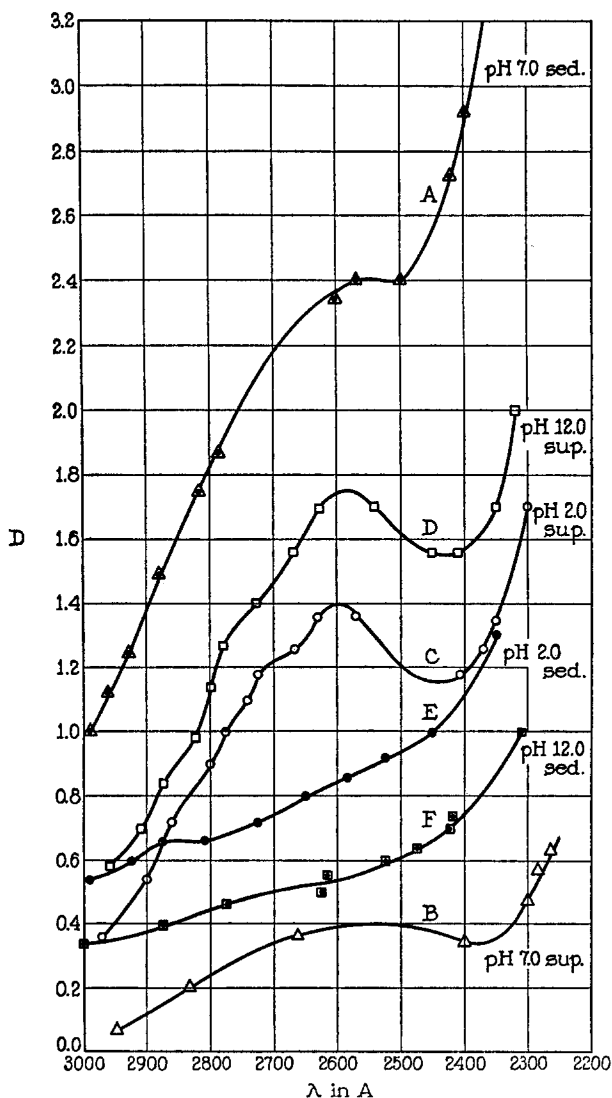
The results are given in Text-fig. 1. Curve B represents the absorption spectrum of the ether fraction, curve C that of the alcohol fraction. It shows that both materials have some absorbing power in the ultraviolet region, but no apparent maximum. At $\lambda 2575$, the combined lipoids account for about 8.5 per cent of the total absorbing power of the complete fraction, as can be seen from Text-fig. 1, curve D, where the ordinate represents the sum of the extinction coefficients of the two lipoids multiplied by the relative concentration of the latter as it occurs in the complete purified fraction. It is apparent that for $\lambda 2900$ to $\lambda 2600$ most of the absorbing power of the purified fraction is a property of the nucleoprotein component of the material (4).

Effect of Acid and Alkali on the Purified Tumor Fraction

Lewis and Michaels have shown that ordinary chicken tumor extracts, adjusted to various hydrogen ion concentrations by means of appropriate buffers, retained their tumor-producing activity for at least 30 minutes in the range between pH 4.0 and pH 12.0, whereas inactivation occurred immediately at, or beyond pH 3.6 and pH 12.5 (6). The results which follow indicate that these findings apply also to the tumor agent in the purified form.

The solubility of the purified tumor fraction was investigated by mixing the stock preparation, in neutral water, with an equal volume of 0.1 M buffer solutions of the proper pH, the final concentration of the substance in the mixture being 0.14 mg. per cc. Under these conditions, the purified fraction forms stable colloidal solutions between pH 7.0 and pH 11.0. Between pH 11.5 and 13, the solution becomes rapidly more transparent. On the acid side, the substance is very sensitive to an increase in hydrogen

ion concentration. Aggregation begins to take place already at pH 6.6, as shown by an increase in the opalescence of the solution. Between pH



TEXT-FIG. 2. Absorption spectra of the purified fraction after treatment with acid or alkali. The ordinates D represent the absorption coefficients in arbitrary units.

4.8 and 2.4 the substance is practically insoluble, the point of minimum solubility being found in the neighborhood of pH 3.5. In solutions more acid than pH 2.4 the material is again more soluble, giving nearly clear solutions at pH 1.0. An interesting correlation appears to exist between

the points of rapid inactivation of the tumor agent and the stability of the purified substance in acid and alkali. Immediate inactivation occurs, on the acid side, at the point of minimum solubility of the purified tumor fraction, *i.e.*, pH 3.5, and on the alkaline side in the region of pH 12.0, where a disintegration of the substance seems to take place. At room temperature, the purified fraction retains its tumor-producing power for at least one hour at pH 4.0 or pH 11 (7). These observations led us to investigate the possible chemical changes produced in the solution, especially at those pH's at which the tumor agent is rapidly inactivated.

To 2 cc. of purified fraction, containing about 1.2 mg. substance per cc., were added 3 cc. of 0.02 N HCl or 3 cc. 0.01 N NaOH solutions, the final pH's being 2.0 and 12.0, respectively. The solutions were neutralized by means of 0.1 N NaOH or 0.1 N HCl, after a period of 1 hour. A sample of the purified fraction was diluted with 0.005 M phosphate buffer and kept at pH 7.0, as control.

In order to demonstrate the possible formation of split products during the treatment with acid or alkali, the neutralized solutions were submitted to high speed centrifugation, at about 18,000 times gravity, for 1 to 2 hours. The sediments were resuspended in neutral buffer and the volume adjusted to that of the supernatant fluids. Both the supernatant fluids and sediments were then examined for their power to absorb ultraviolet light.

The results are shown in Text-fig. 2. Curve A represents the absorption spectrum of the sediment recovered from the sample kept at pH 7.0. It is practically identical with the absorption spectrum of the standard tumor fraction, as illustrated in Text-fig. 1, curve A. Curve B represents the absorption of the corresponding supernatant fluid, showing that absorbing elements were practically absent from the solution. On the other hand, the results were quite different with the samples treated at pH 2 and pH 12. In this case, the supernatant fluids were found to contain large quantities of substances absorbing especially in the region of $\lambda 2600$ (Text-fig. 2, curves C and D), whereas the corresponding sediments, brought back to the original volume, exhibited an absorbing power considerably reduced (see Text-fig. 2, curves E and F). Thus, treatment of the purified fraction at pH 2 or pH 12 liberates certain absorbing constitutents which are no longer sedimentable under a force of 18,000 times gravity for 2 hours. The character of the absorption curve suggests that the substance set free at pH 2 and pH 12 is probably nucleic acid. This is supported by the fact that pH 2 and pH 12 supernatant fluids gave strongly positive tests for pentoses.

Effect of Ultraviolet Light on the Purified Fraction

The susceptibility of the chicken tumor agent to ultraviolet light was demonstrated by a number of workers who endeavored to determine the

relative amount of energy required for complete inactivation (8). Using monochromatic light and plotting the absolute amount of energy against the corresponding wave length, Sturm, Gates, and Murphy were able to establish a curve of inactivation which showed that the most effective region was in the neighborhood of λ2600 (9).

In the following experiments, the purified fraction was exposed to ultraviolet radiations and the absorption spectrum was determined during the course of irradiation to register any change which might take place under these conditions.

The purified fraction was kept in the quartz cell of the spectrograph and exposed to the condensed spark of tungsten steel electrodes, using 6 amperes at 110 volts for periods varying from 5 minutes to 5 hours, the material being left in place for the absorption measurements. During the treatment, the temperature did not rise above 26°C. A control for activity was kept at the same temperature during the experiment.

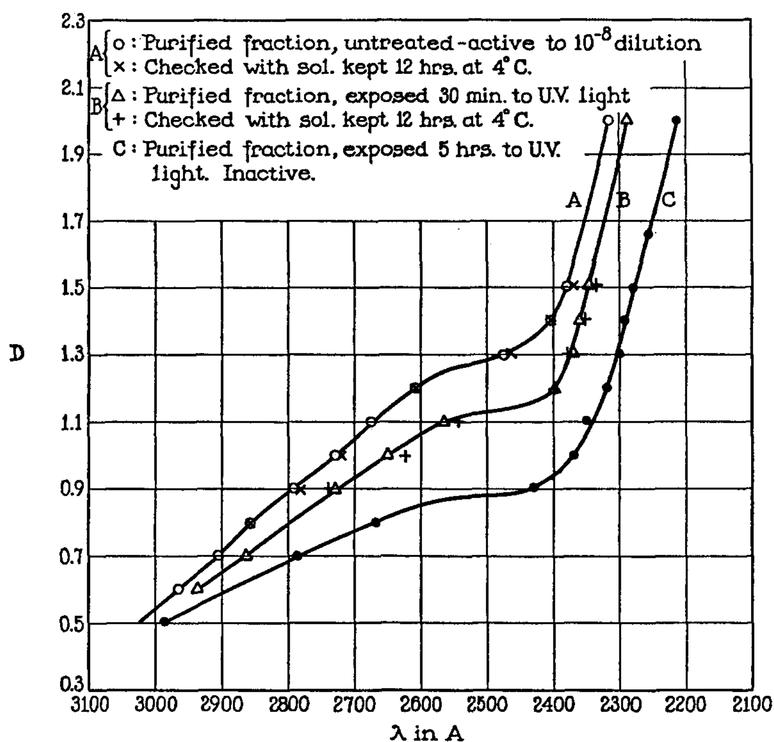
A summary of the results is given in Text-fig. 3. Curve A represents the absorption spectrum of the purified fraction, which contained 0.14 mg. dry substance per cc. and proved active at 10^{-8} dilution. The absorption spectrum of the same solution was redetermined after being kept for about 12 hours at 4°C. and was found unchanged. Curve B represents the absorption spectrum of the purified fraction after 30 minutes exposure to ultraviolet light. The absorbing power of the solution was appreciably decreased, especially in the region between λ2700 and λ2400. At λ2550, the original absorbing power of the solution was reduced by about 12 per cent. Inoculation tests showed that the tumor-producing activity had been completely abolished by this 30 minute irradiation. The material was kept at ice box temperature and the curve was redetermined 12 hours later. No further changes in the curve occurred. Continuous exposure of the purified material to ultraviolet light from a mercury arc, using 6 amperes at 110 volts, at 20 cm. distance for 5 hours, reduced the absorbing power of the purified fraction to about 69 per cent of the original value at λ2550 (see curve C).

Effect of Heat on the Purified Tumor Fraction

Rous and Murphy found that the tumor agent which is present in extracts of tumor desiccates was completely inactivated by heating at 55°C. for 15 minutes, but the tumor-producing power of the material was still demonstrated after heating at 50°C. for the same length of time (10). The following tests indicate that the same temperature range is also effective in bringing about rapid inactivation of the purified tumor agent. Since

mere dilution of the purified fraction with water or buffer solutions often causes an appreciable reduction of the tumor-producing power, relatively concentrated preparations and large volumes of material were used in the heating experiments.

Freshly prepared solutions, containing 1.84×10^{-4} gm. of the purified fraction per cc. in 0.005 M phosphate buffer at pH 7.0, were heated, in 8 cc. lots, to 50° or 65°C.



TEXT-FIG. 3. Effect of ultraviolet radiations on the absorption spectrum of the chicken tumor fraction. The ordinates D express the absorption coefficients in arbitrary units.

for exactly 30 minutes. The samples were enclosed in sealed tubes which were immersed in water baths maintained at the correct temperature. After 30 minutes, the solutions were cooled under running water and stored at ice box temperature until tested. The control solution was kept under the same conditions, but at 4°C., during the experiment. The results of the inoculation tests are given in Table II. It shows that heating the purified fraction 30 minutes at 50°C. had destroyed about 99 per cent of its tumor-producing activity. Heating 30 minutes at 65°C. abolished completely the tumor-producing power of the material.

No important physical changes were produced upon heating at these temperatures, except for a slight decrease in the opalescence of the solu-

tion. The ultraviolet absorption spectrum of the heated solution was determined as usual, and compared with that of the non-heated control. The results are given in Text-fig. 4, which shows that heating the purified fraction to 50° or 65°C. did not modify appreciably its absorption spectrum, the slight differences which are noted being within the limits of experimental error. These observations are in agreement with previous observations (1). Curve A represents the absorption spectrum of the control solution, which proved active at 10^{-8} dilution. Curve B represents the absorption of the solution heated 30 minutes at 50°C., and curve C corresponds to the solution heated 30 minutes at 65°C. As seen from those curves, inactivation of the tumor agent by heat does not affect the ultra-

TABLE II

Effect of Heat on the Tumor-Producing Power of the Purified Fraction

Measurements 18 Days after Inoculation

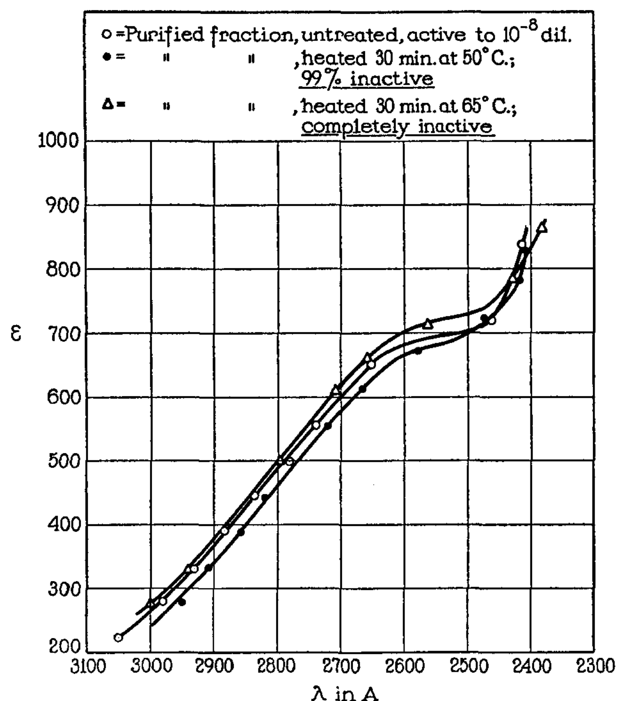
Dilution	Control		30 min. at 50°C.		Control		30 min. at 65°C.	
	Average size of tumors	Takes	Average size of tumors	Takes	Average size of tumors	Takes	Average size of tumors	Takes
	cm.	*per cent*	*cm.*	*per cent*	*cm.*	*per cent*	*cm.*	*per cent*
6×10^{-3}	2.9 × 2.5	100	2.0 × 1.6	100	2.3 × 2.3	100	—	0
6×10^{-4}	2.6 × 2.5	100	1.6 × 1.6	100	2.4 × 2.3	100	—	0
6×10^{-5}	2.0 × 1.8	100	1.2 × 1.1	100	2.0 × 1.0	100	—	0
6×10^{-6}	1.9 × 1.2	100	1.2 × 0.9	100	1.7 × 1.1	100	—	0
6×10^{-7}	1.8 × 1.3	100	—	0	1.2 × 0.8	100	—	0
6×10^{-8}	1.2 × 1.1	100	—	0	1.0 × 0.8	100	—	0

violet absorption spectrum of the purified fraction. Since the specific absorbing power of the purified fraction can be ascribed to the presence of a nucleoprotein, these results could have been expected, because the main absorbing component—nucleic acid—is known to be quite stable at this temperature range. However, the failure to detect a change in the total absorbing power of the solution upon heating did not exclude the possibility of a disruption of the nucleoprotein, an action which may not necessarily affect the structure of the chromophoric group responsible for the ultraviolet absorption power. In order to investigate an effect of this kind, an attempt was made to separate the possible products of dissociation by submitting the heated material to high speed centrifugation.

In these experiments, preparation of the purified material, heating of the solution, and subsequent separation in the high speed centrifuge, was performed the same day in order to avoid possible spontaneous deterioration of the material on standing. 8 cc.

lots of the freshly purified substance were heated for 30 minutes at 50° and 65°C. respectively, a control sample being kept on ice during the same length of time. Immediately after this treatment, the solutions were cooled to 0°C. and then centrifugalized for 2 hours at about 18,000 times gravity. The supernatant fluids were removed and centrifuged once more, at the same speed, for one hour. These twice centrifuged solutions were then examined for their power to absorb ultraviolet light.

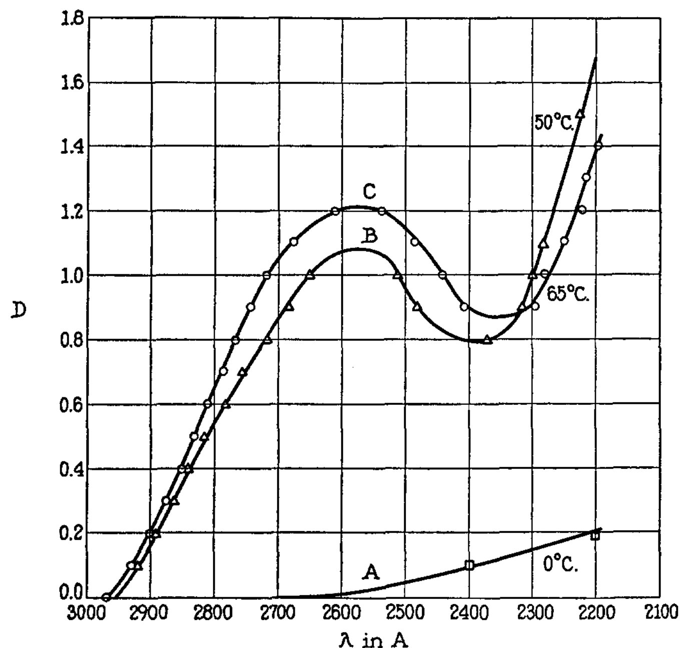
The results are recorded in Text-fig. 5. Curve A represents the absorption spectrum of the supernate derived from the control sample kept at 0°C.



TEXT-FIG. 4. Effect of heat on the ultraviolet absorption spectrum of the purified fraction.

As could have been expected, this solution was completely transparent and contained probably nothing but the phosphate buffer. On the other hand, solutions obtained from the heated materials exhibited a marked absorbing power, with a characteristic maximum at λ2575. Curves B and C correspond to the supernates obtained from the materials heated for 30 minutes at 50°C. and 65°C. respectively. As shown in Table III, the 50° and 65°C. supernates gave negative biuret tests but strongly positive tests for pentoses (Biall's test). The sediments from the first high speed centrifugation were washed once in buffer and resuspended in a volume of

buffer equal to that of the original solution. As shown in Table III, the "heated" sediments retained the protein components of the purified fraction, but lost most of the substance which, in the untreated sample, gave



TEXT-FIG. 5. Effect of heat on the purified tumor fraction; liberation of "nucleic acid" and its separation in the high speed centrifuge. The ordinates D express the absorption coefficient in arbitrary units.

TABLE III

Color Tests for Proteins and Pentoses, on Fractions Derived from Purified Tumor Material, Treated at Various Temperatures

Temperatures at which purified material was treated	Biuret		Biall's	
	Supernate	Sediment	Supernate	Sediment
°C.				
0	−	+	−	++
50	−	+	+±	±
65	−	+	++	±

the characteristic reaction of pentoses. The above observations indicate that heating the tumor fraction at 50°C. for 30 minutes, a treatment which destroys 99 per cent of the tumor-producing activity, causes the separation of an unsedimentable, ultraviolet-absorbing substance. Heating at 65°C.

for the same length of time, a treatment which destroys all the tumor-producing power of the material, causes more of the absorbing element to pass into solution. The characteristic absorption spectrum and color tests suggest that the substance of low molecular weight liberated by heat is nucleic acid.

DISCUSSION

The experiments reported in this paper indicate that the active chicken tumor fraction, isolated by differential centrifugation at high speed, presents a characteristic absorption in the ultraviolet, a maximum being found in the region of $\simeq \lambda 2575$. The findings are in agreement with previous work in which purified fractions obtained by other methods had been found to absorb ultraviolet light in the same manner. Absorption in that region is probably due, to a large extent, to the presence of purine and pyrimidine bases, since as much as 10 to 15 per cent of the material can be isolated in the form of nucleic acid. In the active fraction, the latter is apparently a constituent of a nucleoprotein.

The present observations establish the fact that agents which tend to inactivate the tumor principle will at the same time cause fundamental changes in the constitution of the nucleoprotein. Inactivation of the tumor agent by acid or alkali is accompanied by decomposition of the nucleoprotein into an insoluble protein and free nucleic acid of low molecular weight. Inactivation of the tumor agent by heat likewise corresponds to partial decomposition of the nucleoprotein and the release of nucleic acid into solution. On the other hand, inactivation of the agent by means of ultraviolet light is accompanied by a general decrease in the absorbing power of the purified fraction, this effect resulting probably from important changes brought about in the structure of the nucleoprotein molecule. Heyroth and Loufbourow (11) and Caspersson (12) have found that the ultraviolet absorbing power of nucleic acid is considerably reduced when this substance is submitted to ultraviolet irradiation for some time, the general shape of the curve, however, remaining the same. From Caspersson's results it is apparent that the rate in the decrease of absorption slows down with time of irradiation, and that an equilibrium is probably reached. From our results mentioned above, ultraviolet irradiation is found to affect the purified fraction in a similar manner. The change brought about in the nucleic acid molecule by irradiation has not yet been elucidated. It involves probably saturation or rearrangement of the double bonds in the purine and pyrimidine bases, a change which would influence considerably the property of the substance.

These changes which affect at the same time the tumor agent and the nucleoprotein might be coincidental, and the experiments do not prove that the nucleoprotein found in the purified fraction is necessarily an integral part of the tumor agent. In favor of the view, however, that nucleic acid is an essential constituent of the tumor principle is the fact that the wave length interval in the ultraviolet, which is the most effective in inactivating the tumor agent, coincides with the region of maximum absorption for nucleic acid. In this relation it may be recalled that the inactivation curve of Sturm, Gates, and Murphy (9) is practically reciprocal to the ultraviolet absorption curve of nucleic acid.

One may suppose that the mass of the purified fraction is composed mainly of inert matter, perhaps normal constituents of cells, whereas the part corresponding to the tumor agent is too small to be detected by the analytical methods which we have used. In this case the evidence would still point to a nucleoprotein as a probable constituent of the tumor principle.[4]

SUMMARY

1. The tumor-producing fraction, isolated from Chicken Tumor I by means of differential centrifugation at high speed, has been investigated as regards its power to absorb ultraviolet light. A characteristic absorption spectrum was found, with a maximum at λ2575. The absorbing power of the material in that region was largely due to the presence of nucleic acid, or of a closely related compound.

2. Inactivation of the purified tumor fraction with ultraviolet light depressed the absorbing power of the material, especially in the region of 2600–2500Å. These changes were those which nucleic acid would present under the same conditions.

3. Inactivation of the tumor agent with acid or alkali was accompanied by decomposition of the tumor nucleoprotein and passage of free nucleic acid into solution.

4. Partial or complete inactivation of the tumor agent by heat, at 50° or 65°C., was attended by liberation of nucleic acid of low molecular weight.

[4] Wyckoff (13) observed the presence of large amounts of unsedimentable material in preparations of purified bacteriophage (Northrop, 14), kept at pH 10 for 3 days. At that pH, bacteriophage solutions lose their activity rapidly. Mosaic virus protein decomposes progressively when the pH is raised above 9.0, with the appearance of unsedimentable material in the solution (15). Since these preparations have been shown to contain nucleic acid, it is probable that in the cases quoted the unsedimentable and ultraviolet-absorbing material was largely nucleic acid.

5. The parallelism between tumor-producing activity and the integrity of the tumor ribonucleoprotein suggests that the nucleoprotein may be an essential part of the active principle.

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