*Experiment II.* In this experiment the plastic material was added directly to the solution of 2,4-D. Ten-day-old red kidney bean plants were used as the experimental material, and applications were made by placing a single drop of the solution on the base of one of the primary leaf blades. Pipettes which released 0.05 ml of solution were used for applying the drops. The amount of 2,4-D used in all the solutions remained constant at 1,000 ppm; the amount of plastic material added to the 2,4-D, however, ranged from 0% to 1, 5, and 10%. The plants were harvested 2 weeks after treatment, and the average fresh weight of growth above the primary leaves was recorded for each treatment.

Differences between treatments were noted a few hours after application. It was evident that the response of the bean plants was directly proportional to the concentration of the plastic in the solution. The plants treated with 2,4-D plus a 10% concentration of the plastic showed the greatest degree of inhibition. Those treated with 2,4-D and a 1% concentration of the plastic, although not so severely inhibited as those with the 10% plastic, were nonetheless much more affected than were those receiving only 2,4-D (Table 1). No inhibiting effect was noticed

TABLE 1

FRESH WEIGHT OF BEAN SHOOTS DETERMINED 14 DAYS AFTER TREATMENT WITH 2,4-D SALT AND GEON 31X LATEX*

| Treatment (2,4-D at 1,000 ppm) | Average weight of shoot/plant (gm) | Average weight of shoots on per cent basis 2,4-D = 100 |
|---|---|---|
| Control (untreated) ..... | 2.45 | 144 |
| 2,4-D salt ..... | 1.70 | 100 |
| " " + 1% Geon 31X latex .. | .39 | 23 |
| " " + 5% " " " .. | .25 | 14 |
| " " +10% " " " .. | .23 | 13 |

* The weights represent all growth above the primary leaves.

when the entire plant was sprayed only with the Geon 31X latex in concentrations of 1, 5, and 10%.

*Experiment III.* Since the addition of Geon 31X to a 2,4-D salt solution increased the action of 2,4-D, an experiment was devised to test this combination upon a monocotyledonous plant which ordinarily would be resistant to 2,4-D.

Young oat seedlings approximately 4″ tall were treated with sprays containing 1,000 and 3,000 ppm of 2,4-D. In addition to 2,4-D, some of the sprays contained Geon 31X at 6, 12, and 25%. Plants treated with 2,4-D solutions alone showed no visible effect. Those treated with 2,4-D at 3,000 ppm together with Geon 31X at 25% were severely injured, and almost all the plants died. Plants treated with 2,4-D at 1,000 ppm together with Geon 31X at 25% were also injured, but not as severely; a few of these plants died. Plants treated with 2,4-D at 1,000 and 3,000 ppm together with Geon 31X at 6% were not injured.

The results indicate quite clearly that when Geon 31X latex, a water-dispersible, nontoxic plastic material, is added to 2,4-D either directly in solution or as a coating over plants previously treated with 2,4-D, the effect of the 2,4-D is greatly increased.

The manner in which the plastic material acts to increase the effectiveness of 2,4-D is not known. It is quite possible, however, that the plastic material, which has a low moisture vapor transmission coefficient may seal in the vapors of 2,4-D and hence increase its action because of a more intimate contact with the vapors. It is also possible that the carbon dioxide and oxygen relationships are changed as a result of the coating and hence affect the physiology of the cells, making them more susceptible to 2,4-D injury. The plastic coating may also increase moisture on the cell surface beneath the coating, thus permitting better penetration of the 2,4-D into the plant.

### References


1. HAMNER, C. L., LUCAS, E. H., and SELL, HAROLD M. *Mich. agric. exp. Sta. Quart. Bull.*, 1947, **29**, 337–342.
2. LUCAS, E. H., and HAMNER, C L. *Mich. agric. exp. Sta. Quart. Bull.*, 1947, **29**, 256–262.


## Application of Chromatography to Segregation Studies of the Agent of Chicken Tumor I (Rous Sarcoma Virus)[1]


VERNON T. RILEY

*National Cancer Institute,*
*National Institute of Health, Bethesda, Maryland*


Separation of the virus-like agent of chicken tumor I from the morphologically similar normal constituents of the extract has been attempted by various methods, including flask adsorption, chemical precipitation, selective extraction, and differential centrifugation. The differences in behavior of the tumor agent and some of the nonvirus substances of the extract in the various purification techniques employed was, however, so slight as to make complete separation difficult (*1, 4, 6, 7*).

The achievements of chromatography in the separation of closely related chemical substances (*8, 9*) suggested an exploration of the applicability of the method to the problem of separating morphologically similar subcellular particulates.

Exploratory experiments had indicated that the tumor agent was strongly, but reversibly, adsorbed on diatomaceous silicon dioxide (Celite) in the presence of physiological concentrations of sodium chloride. This combination of relatively inert substances provided the basis for a nondenaturating chromatographic system.

A partially purified chicken tumor extract (*4*) was prepared in 0.9% saline (−0.8 log molar), and equal quantities (50 ml) were adsorbed on identical micro-columns, 8 × 35 mm, consisting of 0.5 gm of Celite as adsorbent.

Seven columns containing the adsorbed agent were developed with 10 ml of 0.9% saline, and each was then eluted with 10 ml of a sodium chloride solution ranging in log molarity from −2 through −5 and terminating

[1] A detailed report will appear in an early issue of the *Journal of the National Cancer Institute.*

with distilled water. The eluate from each column was analyzed for nitrogen and tested in a balanced quantitative bioassay for tumor agent (*2, 3*).

The data summarized in Table 1 and Fig. 1[2] demonstrate the influence of salt concentration in effecting the degree of adsorption or elution of the agent on the silica column. The following experiment was undertaken to determine whether the agent exhibits typical chromatographic banding characteristics:

Ten gm of Celite, deposited from a distilled water suspension, were employed to yield a column 34 × 42.5 mm. A total of 100 ml of partially purified tumor agent extract in 0.9% saline was adsorbed on the column, followed by development with an additional 50 ml of saline. The solid adsorbent column was extruded and sectioned as indicated in Fig. 2. Each segment was suspended in distilled water and poured onto a Whatman No. 1 filter disk in a small Buchner funnel and eluted with 50 ml of cold distilled water. These eluates were tested for nitrogen and tumor agent concentration. The results are indicated in Fig. 2, which shows the relative quantity of agent and nitrogen eluted from each segment. The 50 ml of eluant from the top zone contained a virus concentration of 52% of the starting extract and 8.1% of the nitrogen. In this figure all fractions are compared to the top segment as 100%.

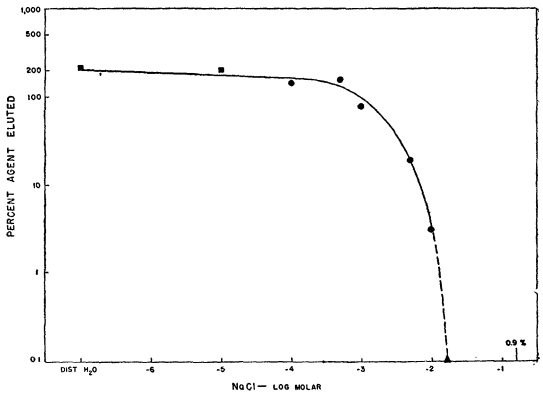


FIG. 1. Elution curve for the chicken tumor agent in the silica-saline chromatographic system.

The data of Table 1 and Fig. 1 contain several implications of biologic and chromatographic interest. In Fig. 1 it is seen that the eluants containing less than 0.001 molar salt concentration release more agent per unit volume than was present in the starting extract. This demonstrates agent concentration on the column under adsorption conditions, followed by a subsequent enrichment of the agent in the eluate. The potency-nitrogen ratios in Table 1 demonstrate the increase in purity of the agent, relative to nitrogen, in the low NaCl eluates. The average was 5.2, with a range from 4.1 to 7.7, or a gain, over the partially purified extract used as starting material, of 310–670%, respectively.

In Fig. 2 it is noted that, in contrast to the regional concentration of the virus in the top of the column, nitrogen unassociated with virus activity is distributed throughout the column and in the filtrate. The agent

[2] The curve in Fig. 1 is the mean of two quantitative experiments. The data of one of these are listed in the table.

TABLE 1

EFFECT OF SALT CONCENTRATION ON QUANTITY OF AGENT AND NITROGEN ELUTED FROM CELITE COLUMNS FOLLOWING DEVELOPMENT

| NaCl concentration of eluants | | Nitrogen (mg/ml) | Nitrogen (%) | Potency* (%) | Standard error of potency estimate | Potency-nitrogen ratio† | Agent recovered (%) |
|---|---|---|---|---|---|---|---|
| Molarity | Log molarity | | | | | | |
| (Starting extract) | | 0.0092 | 100.0 | 100.0 | ..... | 1.0 | 100.0 |
| 0.01 | −2.0 | 0.0017 | 18.5 | 4.0 | + 1.7<br>− 1.2 | 0.2 | 0.8 |
| 0.005 | −2.3 | 0.0013 | 14.1 | 20.4 | + 7.9<br>− 5.7 | 1.5 | 4.1 |
| 0.001 | −3.0 | 0.0017 | 18.5 | 92.0 | +28.7<br>−21.9 | 4.9 | 18.4 |
| 0.0005 | −3.3 | 0.0025 | 27.2 | 138.3 | +55.5<br>−39.6 | 5.0 | 27.7 |
| 0.0001 | −4.0 | 0.0033 | 35.9 | 145.7 | +56.0<br>−40.4 | 4.1 | 29.1 |
| 0.00001 | −5.0 | 0.0024 | 26.1 | 198.6 | +75.7<br>−54.8 | 7.7 | 39.7 |
| Distilled water | | 0.0046 | 50.0 | 210.4 | +82.6<br>−59.3 | 4.2 | 42.1 |

* Expressed as virus concentration compared with that of the partially purified starting extract. The volume of eluate was 1/5 that of the material originally adsorbed.

† Potency-nitrogen ratio equals per cent potency divided by per cent nitrogen to give an approximate index of purity based on nitrogen.

The difference between 4.1 and unity is highly significant statistically; the difference between 7.7 and 4.1 is not. Statistical counsel was provided by Jerome Cornfield.

particulates associated with chicken tumor I are accordingly segregated from some of the nonvirus nitrogen components of the previously purified extract.

With the growing interest in subcellular particulates—both normal and pathological—the need for techniques which permit the separation of these labile entities becomes more pressing. As partially illustrated above, the

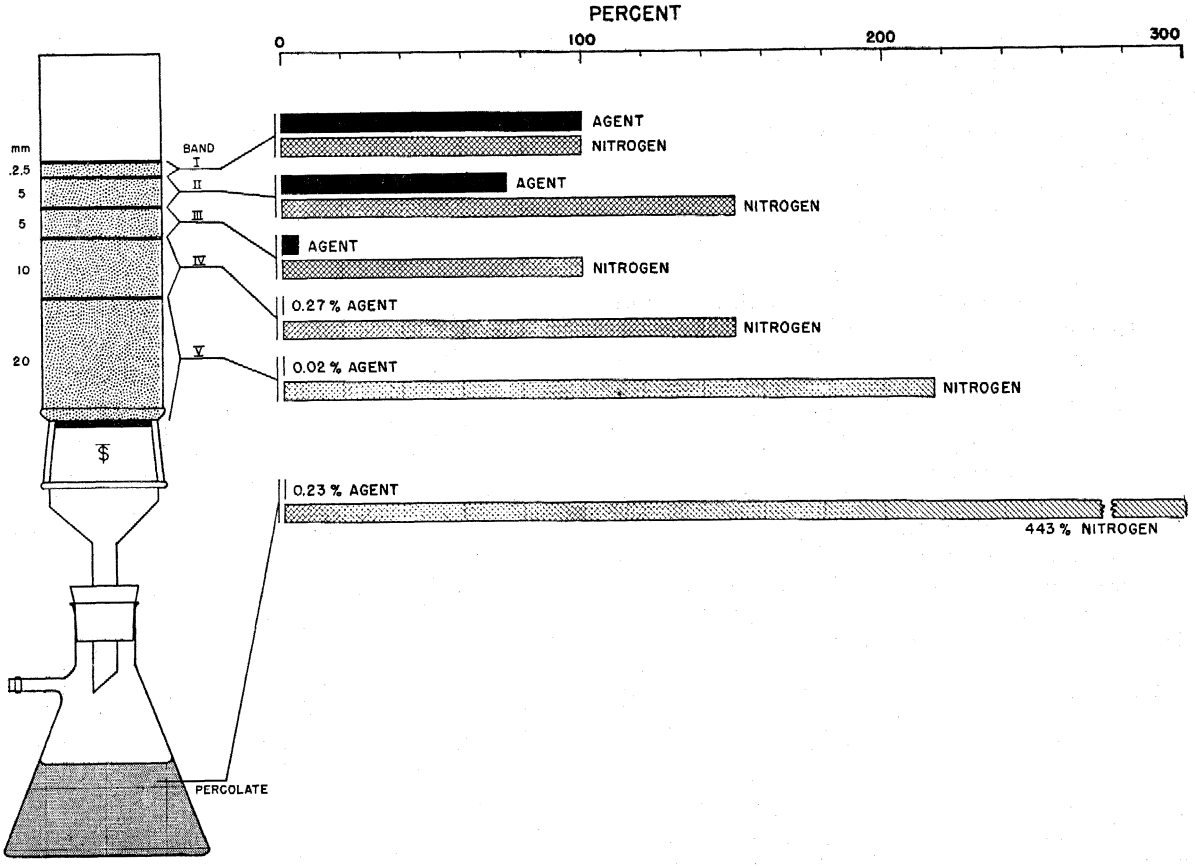


FIG. 2. Segregation of the chicken tumor agent and contaminating nitrogen on silica.

method of chromatographic adsorption may be adaptable not only to the separation of pathologic particulates but, perhaps, also to the study of the "normal" submicroscopic particles.

It is to be noted that in the electron microscope studies of the fractions here reported a high concentration of particles was observed in some of the lower segments of the adsorption columns, where no agent activity was present. These were morphologically similar to the particles observed in the top band, where the agent was concentrated.

It is generally recognized that adsorption phenomena (*5*) of some sort influence the passage of viruses through bacterial filters of the Berkefeld type. Since the reported composition of this filter (*10*) is compressed diatomaceous earth, the adsorption curve in Fig. 1 is of interest. The salt concentration in Locke's, Ringer's, Tyrode's, and other isotonic solutions is considerably in excess of the maximum concentration which permits normal passage of the agent through diatomaceous earth. Approximately 99% of the virus from 50 ml of a highly potent extract was removed by passage through 0.5 gm of diatomaceous earth in the column when the salt concentration was −2 log molar, and none the less than 1% would pass into the filtrate at the physiological level of −0.8 log molar. This is in contrast to the relatively free passage at NaCl concentrations of less than −3 log molar (0.001 M). These results enjoin caution in the interpretation of negative filtration results under conditions which prevented the passage of the highly potent agent here studied.

### References


1. BEARD, J. W. *J. Immunol.*, 1948, **58**, 49–108.
2. BRYAN, W. RAY. *J. nat. Cancer Inst.*, 1946, **6**, 225–237.
3. BRYAN, W. RAY. *J. nat. Cancer Inst.*, 1946, **6**, 373–377.
4. BRYAN, W. RAY, RILEY, VERNON T., DEIHL, GEORGE D., and VOORHEES, VIRGINIA. *J. nat. Cancer Inst.*, 1947, **7**, 447–453.
5. KRAMER, S. P. *J. inf. Dis.*, 1927, **40**, 343.
6. MARKHAM, R. *Parasitology*, 1944, **35**, 173–177.
7. RILEY, VERNON T., CALNAN, DOROTHY, and BRYAN, W. RAY. *J. nat. Cancer Inst.*, 1946, **7**, 93–98.
8. STRAIN, HAROLD H. *Chromatographic adsorption analysis.* New York: Interscience, 1945.
9. ZECHMEISTER, L., and CHOLNOKY, L. *Principles and practice of chromatography.* New York: John Wiley, 1943.
10. ZINSSER, HANS, and BAYNE-JONES, S. *A textbook of bacteriology.* (7th ed.) New York: Appleton-Century, 1935. P. 1106.