situations, is not an absolute requirement for this cytotoxic reaction. This may be true for the lysis of tissue culture cells as well, since human lymphocytes often are cytotoxic for autologous skin fibroblasts, although this reaction may require higher lymphocyte concentrations and longer incubation periods than the allogenic combinations (*10*, *13*). However, the present findings do not indicate that the cytotoxic activity of stimulated lymphocytes is exerted without discrimination in regard to surface structures on the target cells. It cannot be excluded that a recognition step may occur somewhere in the chain of events. Although probably not bound to antibody secretion, this recognition could still be ascribed to receptor molecules on a fraction of the lymphocytes, in some way put into action by stimulation. The receptors could be lymphocyte-associated immunoglobulins or their subunits, with a specificity for target cell determinants. Reaction with the antigens or formation of antigen-immunoglobulin complexes could then trigger the injurious events in many different ways. In an alternative model, the degree of similarity between lymphocyte and target cell surface could determine the outcome of their interaction. In this model, recognition could, for instance, also be explained on the basis of quantitative differences in cell surface concentration or availability of histocompatibility factors. Although this concept would recognize the importance of histocompatibility factors, it would be different from that of allogenic inhibition in its present formulation (*11*), since it would allow for cytotoxic reactions in strictly autologous situations.

The role of lymphocyte cytotoxicity in the course of the various tissue-damaging immune responses mentioned above remains to be established. It would also be important to know how the present findings are related to such phenomena as the emission of macrophage inhibitory activity from sensitized lymphocytes upon contact with antigen, or to the release of leukotactic factors from allogenic lymphocytes kept in mixed culture (*14*). The simplicity of the test system described here should make it well suited for further studies of some of the problems outlined above.

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19 APRIL 1968

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Gas Chromatography for Detection of Viral Infections

Abstract. *Gas chromatograms of serum extracts of dogs inoculated with canine infectious hepatitis virus showed two metabolites not observed in uninoculated animals. Chromatograms of extracts of tissue cultures of dog kidney, inoculated with viruses causing canine hepatitis, herpes, and distemper, and a parainfluenza virus similar to simian virus-5, each showed two or more different metabolites. Two of the distinguishing products from cultures inoculated with hepatitis virus were chromatographically indistinguishable from those found in serums of the animals.*

Recent evidence suggests that gas-chromatographic techniques may provide the basis for a new methodology for the rapid detection and differentiation of free-living microorganisms grown in laboratory media. Distinctive chromatographic patterns have been observed for each of the bacteria examined by procedures designed to detect excreted metabolites that are volatile or can be made volatile, and the products of less than a single bacterial cell may be sensed by chromatographs having suitable detectors (*1*). Distinctive gas-chromatographic signatures can also be obtained when certain cell constituents are examined directly (*2*) or after pyrolysis (*3*). In view of the marked sensitivity of the electron-capture detector to certain metabolites (*4*), we tried to determine whether products appearing in low concentrations as a result of infection could be observed, and whether different pathogenic agents would yield different metabolites.

Healthy mixed-breed dogs, shown by virus-neutralization tests to be susceptible to infectious canine hepatitis virus, were kept in isolation units. After 2 to 4 weeks of observation the animals were inoculated with 1.0 ml of a virus suspension containing $10^{6.3}$ $TCID_{50}$ (tissue-culture infectious dose affecting 50 percent). Dogs were bled for serum prior to inoculation with the virus, and daily or occasionally less frequently thereafter until the animals either died or recovered. Serums were collected from clotted blood within 2 hours of bleeding and stored at −60°C.

The thawed samples of serum (2.0 ml) were treated with 0.2 ml of 5*N* HCl and 1.0 ml of 0.2*M* HCl–KCl buffer, *p*H 2.0, and dried under vacuum; they were then dissolved in pyridine and treated with hexamethyldisilazane and trimethylchlorosilane. A 3-μl portion of the supernatant was injected into an Aerograph model-200 gas chromatograph fitted with electron-capture and flame-ionization detectors. The stainless steel column, 1.83 m long and 3 mm in diameter, was packed with Chromosorb-W treated with hexamethyldisilazane. The liquid phase was 10 percent Carbowax (20-M), and the operating temperatures were 100°, 120°, and 150°C for the column, detectors, and injector, respectively. Nitrogen was the carrier gas.

Serum extracts from all 11 experimental animals contained constituents having retention times (R_t) of about 20, 40, 60, 110, 140, and 190 seconds

Table 1. Responses of cell cultures of dog kidney to canine viruses, as detected by gas chromatography with the electron-capture detector. The peaks were peculiar to the infections indicated; they were absent from all other tissue cultures.

Virus	R_t (sec)	Incubation time (hr)						
		17	25	41	50	65	72	96
		Peak areas (mm^2)						
Infectious hepatitis	95	<10	60	<10	64	<10	440	450
	420	0	236	505	348	400	367	350
	1380	0	0	0	2850	2040	2630	2560
Herpes	220	400	1710	400	4260	5760	4280	<10
	310	235	480	520	394	920	332	352
	1625	0	0	0	0	0	1400	1560
	1905	0	0	0	0	0	500	600
Distemper	350	0	0	0	0	0	294	275
	695	0	0	0	340	650	870	192
	1410	0	0	0	1790	1830	2240	2750
Parainfluenza (SV-5)	55	0	0	0	0	0	1960	1680
	670	0	0	0	0	0	2640	4190
		Degrees of cell degeneration						
Hepatitis		−	−	+	+	+++	++++	++++
Herpes		−	+	+	+	++	++	++++
Distemper		−	−	−	−	−	−	+
Parainfluenza		−	−	−*	−*	−*	++	++++

* Positive hemadsorption noted.

as sensed by the electron-capture detector; the components were found in the extracts from animals whose blood was taken prior to inoculation and at all subsequent samplings. Substances having retention times of about 35, 50, 70, 80, 95, 180, 280, 315, 340, 455, 590, 705, and 1140 seconds appeared in extracts of serums taken from several of the uninoculated dogs; in some instances, one or more of these peaks were absent at the time of inoculation but appeared in one or several of the animals after infection. Such compounds appear to have little or no diagnostic value and probably reflect merely variability in the animal population.

In ten of the 11 animals inoculated with the virus, a peak with R_t of 420 seconds appeared at the first sampling (commonly 24 hours) after inoculation; in some instances the first sampling was on the 3rd day, and such extracts consistently showed the metabolite. In the 11th instance the peak first appeared in chromatograms prepared from serum taken on the 2nd day after inoculation. The substance was not present in extracts of serums of any of the dogs before infection; the peak was clear and distinct, ranging in area from 36 to 1600 mm^2, depending on the animal and the sampling time. This product having an R_t value of 420 seconds appeared in all samples taken from the eight animals that died because of infectious hepatitis, death occurring between the 2nd and 6th days; it had largely or entirely disappeared by the 7th day in the three animals that recovered. Furthermore, a peak having an R_t value of 1380 seconds appeared only in moribund dogs and not in the animals that recovered.

Figure 1, showing results of a typical experiment, reveals few peaks in extracts from blood samples taken from animals before inoculation. Twenty-four hours after inoculation, the metabolite having an R_t value of 420 seconds is evident, while the serum constituent having an R_t value of 1380 seconds

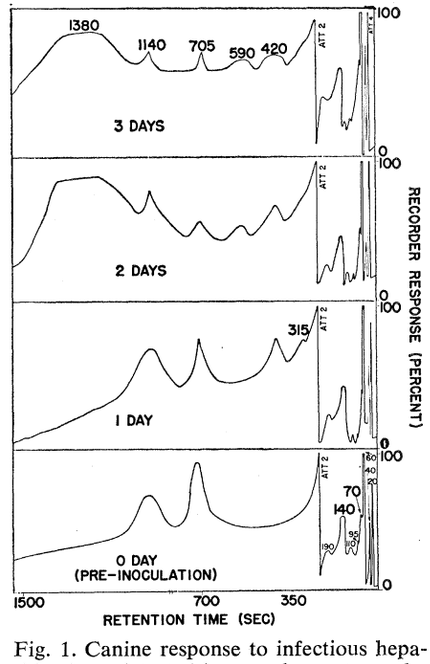

Fig. 1. Canine response to infectious hepatitis virus, detected by gas chromatography of serum samples with the electron-capture detector; *ATT* refers to an attenuation setting of the instrument.

first appears in the sample taken 48 hours after inoculation. Several peaks in the various chromatograms were broad, and precise R_t values could not be established; however, dilution of a sample yielded sharper peaks and more-precise R_t values.

These products may result from a relatively nonspecific change in the physiology of the animal because of the physiological stress arising from the virus infection. Alternatively, the constituents may be uniquely associated with infections induced by this virus; if this is so, diagnosis of the disease may be based on the presence of these peaks. To test whether the generation of these products was associated with the infectious hepatitis virus originally employed, tissue culture experiments were conducted with the hepatitis virus, strain F-205 of the canine herpes virus (*5*), the Rockborn strain of the canine distemper virus (*6*), and the parainfluenza virus resembling simian virus-5 (SV-5) (*7*). Primary cultures of kidney cells from beagle pups, reared in sterile isolation and aged from 3 to 5 weeks, were prepared with Earle's saline solution, 0.5 percent lactalbumin hydrolyzate, and 10 percent newborn-lamb serum as a growth medium. When monolayers had formed, usually after 3 to 4 days, the cultures were washed once with the saline solution, and 0.2 ml of virus preparation was inoculated into each of 25 cultures. After a 2-hour adsorption period, the cultures were washed with the saline, and 1.5 ml of normal dog serum was added to each culture. The fluid portion was harvested from three randomly selected cultures immediately after virus inoculation and at regular intervals thereafter. Uninoculated cultures were harvested at each sampling time.

All cultures were examined for cytopathic effects (Table 1). Cell degeneration was observed 41 hours after inoculation with infectious hepatitis virus; 25 hours, with herpes virus; and 96 hours, with distemper virus. With the parainfluenza virus resembling SV-5, hemadsorption of guinea pig erythrocytes was evident 41 hours after inoculation; cell degeneration, after 72 hours.

Samples to be chromatographed were centrifuged for 10 minutes at 1,000*g*, the supernatant was rapidly frozen, and the sample was stored at −60°C pending the analysis described. Peaks having retention times of 20, 40, 65, 115, 140, 380, 705, and 1040 seconds were present in chromatograms of both uninocu-

lated and inoculated cultures. Other constituents were found in infected and not in uninfected tissue cultures, but their occurrence was not unique to any one of the four virus-infected cultures—for example, metabolites having retention times of 80, 125, 165, 200, 265, 290, 510, 565, and 1130 seconds. However, as shown in Table 1, two compounds observed in the cell cultures of dog kidney had the same retention times as compounds found in the serums of animals inoculated with the hepatitis virus—R_t values of 420 and 1380 seconds; these two metabolites were not present in cultures receiving any of the other viruses investigated. Four distinctive peaks were noted in cultures inoculated with the herpes virus, and three different products were observed in distemper-infected cultures, while two differentiating peaks were found in cultures receiving the parainfluenza virus.

The degree of specificity of the products of these particular virus infections remains unknown; more viruses must be investigated. However, correlation between the appearance of products having R_t values of 420 and 1380 seconds and infection with infectious hepatitis is suggested by the observations that the same two compounds were present in all 11 animals infected with the virus, metabolites having identical chromatographic characteristics were observed in tissue cultures receiving this virus but in none of the others, and the disappearance of these metabolites coincided with recovery of the animals.

If a specific or possibly unique metabolite is produced in minute amounts in animals as a consequence of or prelude to a particular infection, ultrasensitive gas-chromatographic methods may be able to detect these distinguishing substances. By means of such highly sensitive procedures, it may then be possible to perform an early or rapid diagnosis of microbial infections of animals and even humans.

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Electrically Supported Column of Liquid

Abstract. *Application of an electric field normal to the interface of certain liquids causes a liquid column of uniform diameter to be formed parallel to the electric field. A column of amyl alcohol supported in air by a high voltage was investigated experimentally; the diameter of the column varied as the voltage raised to the 3.5 power.*

Recently the various phenomena (such as pumping, stirring, electroconvection, production of a spray) associated with the interaction of an electric field with a dielectric fluid have attracted much attention (*1*). During investigation of the stability of a fluid interface in the presence of an electrostatic field (*1*) it was observed that certain liquids form columns, of uniform diameters, extending from the surfaces of the liquids to the upper electrode (Fig. 1). To the best of my knowledge, electrical formation and support of a liquid column had never been mentioned (*2*, *3*).

A strong electrostatic field was applied (Fig. 2) normal to various liquids in order to determine which liquids would form a column surrounded by air and extending from the liquid surface to the upper electrode. For initiation of the column, the upper electrode was first lowered until distance h was quite small. A liquid having moderate electrical conductivity, such as tap water, underwent much surface activity (formation of surface waves) but formed no column because the air between the liquid surface and the upper electrode broke down. A liquid having lower conductivity, such as ethylene glycol (10^{-5} mho/m), formed a peak extending from the liquid surface to the upper electrode. A liquid low in conductivity, such as fuel oil, experienced strong pumping in that a fountain of liquid was continuously circulated between the liquid surface and the upper electrode.

Of the various liquids tested, amyl alcohol formed easily the longest (up to 3 cm) uniform-diameter column in air. At a relatively low field strength (preceding collapse of the column) the top of the column narrowed down; at a relatively high field strength (when h was quite small) the top of the column widened and resembled in shape the base of the column. Thus the experimental investigation with amyl alcohol excluded the cases of high and low field strengths.

The height of the column was measured by displacement of the string from its reference point (Fig. 2); this height was used also to calculate the average electric field: $E = \psi_A/h$. The relative diameter of the column (the diameter

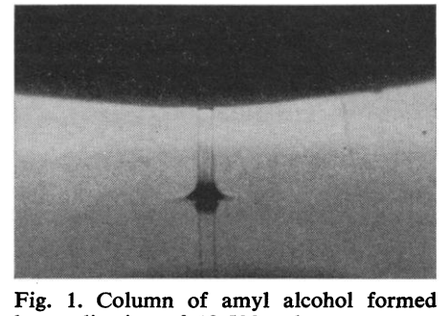

Fig. 1. Column of amyl alcohol formed by application of 12,500 volts.

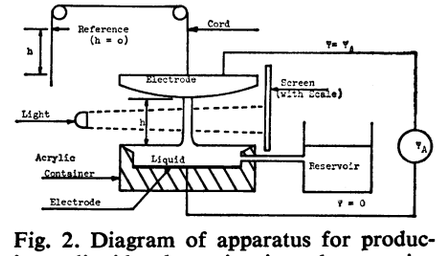

Fig. 2. Diagram of apparatus for producing a liquid column in air and measuring its diameter and height.