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Effect of Interaction of RNA and Polyglucose on Their Solubility in Water-Alcohol Mixtures.* (32453)

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Extraction of tissue with a mixture of phenol, deoxycholate and NaCl yields nucleic acids (DNA and RNA) which differ in biologic activity and physical properties from other preparations(1). The nucleic acid obtained by this method from viral infected tissue is infectious and unlike other preparations(2) the RNA is less soluble than DNA. The RNA is precipitated from aqueous salt solutions by 20% ethanol while 50% is required for DNA(3). The RNA so obtained by alcohol precipitation of the aqueous phase of the extraction contains much polyglucose (CHO) of 2-4 $\times$ 10^6 molecular weight(4). In view of reports elsewhere that the infectivity of nucleic acid isolated from poliovirus(5), gastroenteritis virus(6), or simian virus 40(7) is markedly enhanced by the presence of polysaccharide, it was considered possible that both the biologic activity and unusual solubility of RNA might not result entirely from its intrinsic properties but as a consequence of its interaction in solution with the CHO which accompanies it upon isolation. In the present study, experiments were designed to determine whether such interaction does occur with a resulting alteration in the physical properties of the CHO and RNA.

Methods. HeLa cells were grown in monolayers in Roux bottles with Eagle's medium (8). After removal of the growth medium and rinsing of the cultures with phosphate buffered saline (pH 7.2) 10 ml of 1 M NaCl containing 10% deoxycholate were added to remove the cells from the glass. The bottles were next rinsed with 10 ml of water-saturated phenol which was then combined with the 1 M NaCl. The mixture was shaken mechanically at room temperature. After separation of the aqueous phase, a second extraction with phenol was performed. The dissolved phenol was removed from the aqueous phase by ether extraction and the remaining ether removed by a stream of nitrogen gas. The procedure is essentially as described by Colter *et al*(1) for extraction of nucleic acids. The resulting aqueous phase contained DNA, RNA and CHO (polyglucose). The RNA and CHO were precipitated together free of DNA from the molar salt solution with 20% ethanol at 4°C by standing overnight, and then dissolved in 1 M NaCl(3).

The RNA content was determined by the orcinol method(9) and the CHO with a diphenylamine reagent(10,11).

Experimental and results. The RNA and CHO in a 1 M NaCl solution (prepared as described under *Methods* were separated by careful acid precipitation of the RNA. The two components were separately redissolved. A reconstituted mixture of the two was also prepared and the 3 samples, after being adjusted to equivalent concentrations in 1 M NaCl, were compared with a reprecipitated aliquot of the original mixture in regard to the solubility in 20% ethanol of the RNA and CHO constituents. Details of the procedure are as follows:

Sufficient 40% trichloroacetic acid (TCA) was added to 2 ml of a 1 M NaCl solution containing RNA and CHO (described under *Methods*) to give a final concentration of 5%. The resulting precipitate was washed

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with cold 5% TCA, dissolved at once in 1 ml phosphate buffered saline, pH 7.4 (PBS) and diluted with 1 ml of 2 M NaCl. Five volumes of absolute ethanol were added to the supernatant obtained from the original acid precipitation in order to precipitate the CHO which is soluble at acid pH. The CHO precipitate obtained after the alcohol solution had stood at 4°C overnight was dissolved in 1 ml PBS and diluted with 1 ml of 2 M NaCl. Next, the RNA and CHO were precipitated from 1 ml of the original solution with 20% ethanol, 4°C, overnight and redissolved in 1 ml PBS and diluted with 1 ml of 2 M NaCl. One ml of both RNA and CHO solutions were combined. In addition, one ml of each was diluted with 1 ml of 1 M NaCl. Thus the concentrations of RNA in the reprecipitated original sample, in the reconstituted mixture, and in the pure RNA were the same while the concentrations of CHO were the same in the original, the mixture, and the pure CHO. To the 4 samples, 50% ethanol was added to give a final concentration of 20%. After 12 hours at 4°C, all samples were examined and centrifuged. Precipitates were obtained with all samples except the isolated CHO. These precipitates were separated from the supernatant portions and dissolved in 2 ml of 1 M NaCl. Dissolved pellets and supernatants were assayed for CHO and RNA. The orcinol determinations were corrected for CHO when the latter was present and the resulting data recording in Table I.

TABLE I. Precipitability of RNA and CHO Alone and in Combination by 20% Ethanol.

Sample	Supernatant* CHO	Supernatant* RNA	Precipitate* CHO	Precipitate* RNA
Original†	5.78	9.17	70.8	167
Mixture‡	20.6	5.78	61.4	131
RNA§	—	26.3	—	131
CHO§	68.6	—	—	—

* All values are expressed in μg.
† The original sample is a 1 M NaCl solution of CHO and RNA obtained by dissolving a 20% ethanol co-precipitate recovered from the aqueous phase of a phenol extraction of HeLa cells.
‡ The mixture is a combination of the isolated RNA and CHO in the same proportions as the original sample.
§ RNA and CHO were isolated from an equivalent portion of the original sample.

In Table I it is seen that the CHO in the original mixture, obtained by 20% ethanol precipitation, was reprecipitated almost completely with 20% ethanol. That which had been isolated by the action of TCA and 80% ethanol was not reprecipitated by 20% alcohol unless mixed with isolated RNA. Further, isolated RNA was reprecipitated largely with 20% ethanol but not so completely as in the original sample or as in the mixture reconstituted with isolated CHO. The solubility of each is affected by the presence of the other, particularly if the RNA is partially denatured.

In a series of experiments the precipitability in 20% ethanol of acid isolated RNA varied from 22 to 83%. When RNA was twice precipitated by TCA, its solubility in 20% ethanol was greatly increased. However, with a single preparation of isolated RNA, the fraction precipitating in 20% ethanol was not greatly affected when the concentration of RNA was varied from 105 to 884 $\mu g/ml$ (Table II).

Since isolated CHO would precipitate only in the presence of RNA the capacity of the

TABLE II. Variation in Alcohol Solubility of Several Preparations of RNA Isolated by Acid Precipitation.

Preparation	RNA ($\mu g/ml$*) in 1 M NaCl	RNA ($\mu g/ml$†) in 20% Et	% RNA Precipitated
1	403	76	81
2	157	26	83
3	82	64	22
4	105	58	45
4	123	63	51
4	295	179	39
4	884	447	50

* Concentration of TCA isolated RNA in sample.
† Concentration of RNA remaining in solution after 20% ethanol precipitation, 4°C, 12 hr.

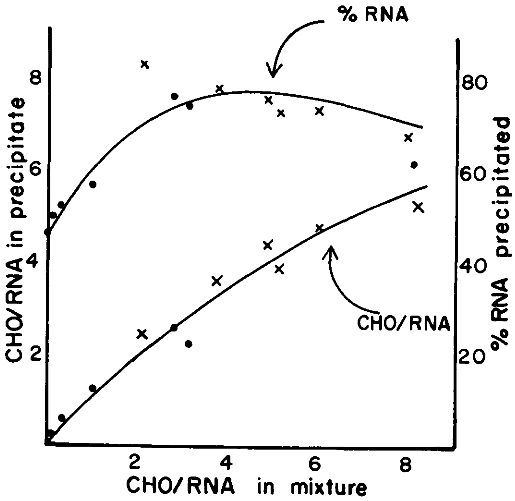

FIG. 1. Effect of the proportion of CHO and RNA in a mixture upon the composition of that which precipitates from 20% ethanol. Weight ratios of CHO/RNA are used. Data are from two comparable experiments distinguished by the dots and crosses.

latter to effect the change in CHO solubility was determined. Mixtures of the two were reconstituted in varying proportions and the composition of the precipitate which occurred in 20% ethanol determined. In Fig. 1 the ratios of CHO to RNA in the various precipitates are plotted against the corresponding ratios of CHO to RNA in the mixture before ethanol addition. The composition of the precipitates was affected by the proportions of the original mixture but not by the absolute concentrations when these were varied over an 8-fold range. Also recorded is the percent of the total RNA which could be precipitated. This increased from 45% to a maximum of 80% which was obtained with a CHO/RNA ratio of 3. Although a combining capacity of 6 could be reached with an original mixture of 8 to 1, the remaining 20% RNA was not precipitated. The slope of the precipitation curve decreased with increasing CHO/RNA ratios in the mixture, however, the maximum combining capacity cannot be determined from the present data.

Discussion. The water-alcohol solubility of RNA extracted with phenol is low in relation to DNA or to other preparations of RNA(3). The data of Table I suggest that this is a result in part of its native state and in part to the presence of CHO in the preparation. The portion of the acid isolated RNA which is soluble in 20% ethanol could be accounted for by a second species of RNA in the original extract. However, since it was observed to increase upon repeated acid precipitation, it must result in part from denaturation of an original insoluble molecular type. This more soluble portion of RNA is, however, precipitated by 20% ethanol in the presence of CHO. The CHO, although soluble in 20% ethanol when alone, is found also in the precipitate (Fig. 1) when mixed with RNA. Thus the precipitation of CHO from the original extract results from its interaction with insoluble and possibly soluble RNA. Such an interaction would be expected to affect the interpretation of observed physical properties of RNA as sedimentation or light scattering. Indeed, polyglucose could be the cytoplasmic component shown by others(12) to produce aberrant sedimentation of RNA.

The absence of highly charged reactive groups in the polyglucose(4) suggests that its interaction with RNA is through hydrogen bonding. Such interaction would explain why the polyglucose is not distributed between RNA and DNA as these are successively precipitated with increasing alcohol concentrations from a three component extract. Many sites for hydrogen bonding are internally occupied as a result of the double helical structure of DNA, thus making it less reactive. In fact, preliminary experiments evidenced interaction between isolated polyglucose and heat denatured DNA but not native DNA. Reactivity between such a naturally occurring high molecular weight structure and sites important in the function of nucleic acids suggests a possible role of the polyglucose in the control of nucleic acid function.

Summary. Phenol extracted RNA, unlike other preparations, is less soluble than DNA in aqueous-alcohol mixtures. Further, the RNA so prepared is associated with a high molecular weight polyglucose (CHO). These components were separated by acid precipitation and their solubility determined separately and in reconstituted mixtures. RNA, free of CHO, remains largely precipitable with 20% ethanol. Isolated CHO precipitates

from 20% ethanol only upon addition of RNA which can effect the precipitation of six times its weight of CHO. Denatured fractions of RNA, soluble under these conditions, also precipitate in the presence of CHO. Despite the interaction of these macromolecules in solution, the unusual solubility properties of phenol extracted RNA seem to be a consequence of its native state rather than the presence of CHO.

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Effect of Hyperthermia on Blood Platelets in Male Rats. (32454)

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Previous reports on the effect of various types of stressors on platelets have indicated that the trauma of surgery(1) and hemorrhage(2) as well as hypothermia(3) have increased the number of platelets in the peripheral blood of experimental animals. Though exposure to cold has caused thrombocytosis (3) as well as thrombocytopenia(4), information on the effect of heat stress on platelet counts has been lacking. This investigation was undertaken to determine whether short term exposure of hyperthermia could alter the platelet counts of rats.

Methods and materials. Forty male Wistar rats (150-300 g) were divided into 2 equal groups and maintained before use in an air-conditioned animal room (26°C) with 14 hours of artificial lighting and were fed Purina laboratory chow and water *ad libitum*. On the day of exposure to heat, body weights and platelet counts of all animals were recorded (initial values) before placing them in individual glass cages. Experimental animals were placed in an incubator with an ambient temperature of 55°-57°C for 15 minutes while control animals remained at room temperature in the laboratory. After the 15 minute period (0 hour), the body weights and platelet counts were again determined in both groups. The animals were kept in the individual cages in the laboratory without access to food or water for the next 4 hours, and body weights and platelet counts were determined hourly (day 1). They were subsequently returned to the colony cages in the animal room and weights and platelet counts were recorded daily for the next 7 days (through day 8). Data were analyzed for changes from the animals' own initial (control) values as well as for differences between the average changes for both groups.

Tail vein blood was collected in Trenner automatic red blood cell pipettes (1:200) and diluted with 1% ammonium oxalate solution which had been regularly checked for contamination. A collodion seal permitted repeated collection of blood from the same incision. Pipettes were shaken for 5 minutes in

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