was 17 cc, and the third, derived from the colorless ice block, was 58 cc.

Quantitative determinations of proteins, albumin, chlorides, cholesterol and isoagglutinins were performed on the 3 fractions and on a sample of the original plasma. The results are presented in Table I. From these data, it can be seen that in the first fraction all the solid plasma constituents are contained in high concentration.

The percentage of each plasma constituent as compared with that of the original plasma are given in Fig. 1. From these, it is apparent that in the first fraction which represents only 25% of the original fluid volume, 70% of the proteins and chlorides is present, while in the third fraction, 58% of the original volume, only 8% of the proteins and 18% of the chlorides are present. Centrifugation of a frozen sample of plasma yielded similar results more rapidly and with a more distinct separation of the layers.

From the above findings, it is felt that these rapid and simple methods which do not require the use of chemicals may be of value in the concentration of plasma and serum constituents.

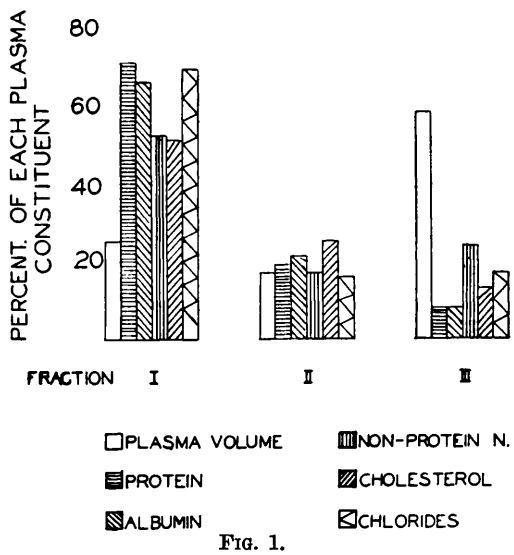

FIG. 1.

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Urinary Excretion of Acid-Decomposable Hydrocarbon Precursors Following Administration of Polycyclic Hydrocarbons.

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In 1934 it was shown[1] that when naphthalene is administered to rabbits there is excreted in the urine a compound which is decomposed by acid, but not by alkali, to yield naphthalene. Later work[2] revealed that such a compound is present in the urine of the rat and other species after the administration of naphthalene. Anthracene[3] and phenanthrene[4] when administered to rats and rabbits give rise to the excretion of compounds which yield anthracene and phenanthrene respectively when decomposed by acid. None of these hydrocarbon precursors has been isolated from urine. The present work was undertaken in order to obtain quantitative data on the excretion of these compounds and to study the question of whether analogous compounds are formed in the organism from other polycyclic hydrocarbons, namely, acenaphthene, chrysene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene and methylcholanthrene.

An isolation procedure was developed in

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1 Bourne, M. C., and Young, L., *Biochem. J.*, 1934, **28**, 803.

2 Stekol, J., *J. Biol. Chem.*, 1935, **110**, 463; 1935, **113**, 675; 1937, **121**, 87.

3 Boyland, E., and Levi, A. A., *Biochem. J.*, 1936, **30**, 1225.

4 Young, L., and Britton, A., unpublished observations.

order to determine the amount of hydrocarbon liberated by the acid decomposition of urine obtained from rats after hydrocarbon administration. The sample of urine (50 ml) was made alkaline to litmus and extracted in a continuous extractor for 6 to 8 hours with ether (150 ml). This ether extract was evaporated and the residue was tested in order to determine whether it contained any acid-decomposable hydrocarbon precursor. With none of the hydrocarbons studied was the preliminary ether extract of the alkaline urine found to contain acid-decomposable hydrocarbon precursor. After the preliminary extraction with ether the urine was acidified with hydrochloric acid to a pH measured by a Coleman pH electrometer and allowed to stand at room temperature. The urine was then made alkaline to litmus with sodium hydroxide and re-extracted for 10 hours with ether (150 ml). This ether extract was washed first with 2N HCl, then with 2N NaOH, and finally with water. It was dried over anhydrous calcium chloride, filtered into a weighed flask and evaporated. The residue so obtained was dried in a desiccator and weighed. The weight of residue less the "blank value" for the urine of undosed rats represented the weight of hydrocarbon liberated by decomposition of the precursor compound. In a series of experiments with 50 ml samples of normal rat urine the average "blank value" was found to be 2.2 mg and in no experiment did it exceed 3.0 mg. In some of the experiments described later hydrocarbons were administered in liquid paraffin. Control experiments in which rats were dosed with liquid paraffin showed that the administration of this substance did not influence the "blank value" of the urine to a detectable extent. The efficiency of the isolation procedure was investigated in a series of experiments in each of which a known amount (10-50 mg) of hydrocarbon for which the procedure was to be used was added to a sample of rat urine which had previously been rendered alkaline and extracted with ether. Under these conditions the recovery figures obtained ranged from 94 to 102% of the amount of hydrocarbon added.

A study of the decomposition of the naphthalene precursor present in the urine of rats dosed with naphthalene showed that at room temperature the optimal acidity for the liberation of naphthalene was pH 1.5 to 2.5 and that under these conditions the amount of naphthalene liberated did not increase after 8 hours. Determinations were then made of the amounts of naphthalene liberated in periods of not less than 8 hours at pH 1.5 to 2.5 at room temperature from the urines of groups of male white rats dosed with known amounts of naphthalene. The rats used weighed from 200 to 300 g each. Two methods of administering the hydrocarbon were employed. In the first of these the hydrocarbon was dissolved in warm liquid paraffin and given by stomach tube. In the second method of administration the rats were fed for 2 one-hour periods each day in feeding cages on the stock colony diet to which the hydrocarbon had been added to the extent of 1%. Significant amounts of naphthalene precursor were not detected in the urine excreted later than two days after the completion of dosing. In order to determine the proportion of the dose of naphthalene which was excreted as the acid-decomposable hydrocarbon precursor, analyses were made of the urine collected during the dosing period and for three days after the completion of dosing. Experiments similar to those described above were also performed with phenanthrene and with anthracene. The results obtained in the experiments with naphthalene, phenanthrene and anthracene are summarized in Table I.

Experiments were performed in which 2 male rabbits, each weighing approximately 2 kg, were dosed with naphthalene. Each rabbit received by stomach tube a single dose of 2 g of naphthalene dissolved in warm liquid paraffin. The urine from each rabbit was collected daily for 3 days after the administration of the naphthalene, and the amount of naphthalene liberated from the urine under the optimal conditions described above was determined. The maximum liberation of naphthalene occurred in the urine collected on the first day after dosing. Traces of naphthalene were liberated from the urine excreted on the third day after dosing. In the urine collected in the 3-day period after dosing 5.9% of the naphthalene administered was isolated in the experi-

TABLE I.
Amounts of Hydrocarbon Liberated at pH 1.5-2.5 at Room Temperature in Periods of Not Less than 8 Hours from Rat Urine Excreted Following Administration of Naphthalene, Phenanthrene or Anthracene.

Hydrocarbon	No. of rats in group	Mode of administration	Mg of hydrocarbon admin.	Mg of hydrocarbon liberated	% of hydrocarbon intake liberated from urine
Naphthalene	8	Stomach tube	1600	224	14.0
"	8	" "	800	121	15.1
"	4	Mixed in diet	535	89	16.6
"	4	" " "	770	98	12.7
Phenanthrene	4	Stomach tube	800	48	6.0
"	4	" "	800	43	5.4
"	4	Mixed in diet	720	28	3.9
"	4	" " "	685	50	7.3
Anthracene	4	Stomach tube	800	18	2.3
"	4	" "	800	26	3.3
"	4	Mixed in diet	845	23	2.7
"	4	" " "	830	21	2.5

ment with one rabbit and 8.5% in the experiment with the other.

Experiments were also performed with the non-carcinogenic hydrocarbons acenaphthene and chrysene, and the carcinogenic hydrocarbons 3,4-benzpyrene, 1,2,5,6-dibenzanthracene and methylcholanthrene. These hydrocarbons were administered singly to groups of male white rats by admixture in the stock colony diet to the extent of 1%. The conditions of the experiments were similar to those described above in which naphthalene was administered to rats by this method. After the adequacy of the isolation procedure had been established for each hydrocarbon the urines were subjected to the conditions which were found to be optimal for the breakdown of the naphthalene precursor excreted by rats receiving naphthalene. The application of the isolation procedure to the acidified urines yielded small amounts of material which in every experiment corresponded to less than 1% of the hydrocarbon administered. In no case, however, when the residues from a series of experiments with a given hydrocarbon were combined and examined was it possible to detect the presence of hydrocarbon. In no experiment was hydrocarbon found on acidification of the residue obtained by evaporation of the preliminary ether extract of the alkaline urine, and the failure to isolate hydrocarbons from the acidified urines was therefore not due to removal of the precursor compounds by the preliminary ether extraction of the urines. It therefore appears that the urines of the rats dosed with acenaphthene, chrysene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene or methylcholanthrene either did not contain hydrocarbon precursors decomposable under the conditions used or that such precursors were present in smaller amounts than could be detected by the methods employed.

Summary. After the administration of naphthalene to rats there is excreted in the urine a compound which can be decomposed by acid to yield naphthalene. The optimal acidity for the decomposition of this compound at room temperature was found to be pH 1.5-2.5, and under these conditions the amounts of naphthalene liberated did not increase after 8 hours. When the urines obtained after the administration of naphthalene to rats by stomach tube or by admixture in the diet were acidified under the optimal conditions described above, from 12.7 to 16.6% of the naphthalene administered was liberated. In similar experiments with phenanthrene and with anthracene the acidification of the urines obtained resulted in the liberation of from 3.9 to 7.3% of the phenanthrene and from 2.3 to 3.3% of the anthracene administered. No liberation of hydrocarbon was detected when the urines excreted by rats on diets containing acenaphthene, chrysene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene or methylcholanthrene were acidified to pH 1.5-2.5. If

the ingestion of these hydrocarbons by rats is followed by the excretion of acid-decomposable hydrocarbon precursors either such compounds are excreted in very small amounts or their decomposition occurs under conditions which differ from those which bring about the breakdown of the naphthalene precursor excreted after the administration of naphthalene.

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Antigenic Relationships of the Species *Shigella dispar.*

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Castellani[1] isolated from the feces of patients with clinical dysentery a bacterium which he named *"Bacillus ceylonensis B."* Five years later[2] he reported the isolation from normal human stools of *Bacillus madampensis.* Both organisms were gram-negative, nonmotile rods, fermenting many carbohydrates, including lactose, with production of acid only, and producing indol. *Bacillus ceylonensis B* fermented dulcitol, whereas *Bacillus madampensis* did not. Both fermented sucrose. Andrewes[3] included these 2 species, together with some other lactose-fermenting dysentery-like varieties, in his *"Bacillus dispar."* Current terminology places these microörganisms in the genus *Shigella.*

Castellani stated that *Bacillus ceylonensis B* was a serologically homogeneous species. Glynn and Starkey,[4] working with 15 strains, reported *Shigella dispar* antigenically heterogeneous. The present investigation was undertaken to study further the antigenic structure of strains designated *Shigella dispar* or *Shigella ceylonensis.*

Thirty-seven strains of bacteria are included in this preliminary report. Five were received from the American Type Culture Collection; 10 were received from Dr. K. M. Wheeler, and were isolated in Connecticut within the past 4 years; 3 were isolated in Texas in 1942, and were received from Dr. MacDonald Fulton; two were isolated recently in Rhode Island by Dr. C. A. Stuart; the remaining 17 were isolated in a number of different laboratories along the Atlantic seaboard. Eleven strains of *Sh. ceylonensis* were received, and 26 strains of *Sh. dispar.*

All 37 strains produced acid from dextrose, lactose, maltose, and mannitol; failed to ferment salicin, and were indol positive. Further fermentation reactions are noted in Table I.

Antiserums were prepared for 2 strains of *Sh. dispar* and one of *Sh. ceylonensis.* Table I presents a summary of the results obtained when the 37 strains were tested for agglutination in the various antiserums. The titers stated are those for the type strains listed, but all organisms grouped together behaved similarly.

It is apparent that the 11 strains of *Sh. ceylonensis* are serologically identical or closely related to *Sh. dispar* type 205. Most of the type 205 strains were pigmented, the color varying from a faint yellowish-orange to a deep orange. None of the *Sh. ceylonensis* strains were pigmented, but there appears to be no correlation between pigment production and antigenic structure.

Two other antigenic types, 171 and 221, are evident. Thus, there are 3 well defined serological groups, the major group including 21 of the 26 strains of *Sh. dispar,* together with all 11 strains which were designated *Sh. ceylonensis* on the basis of their fermentation reactions.

This grouping of the strains is confirmed by adsorption experiments. Antiserums in 1:100 dilution were adsorbed twice for one hour at

1 Castellani, A., *J. Hyg.*, 1907, **7**, 1.

2 Castellani, A., *C. f. Bakt.*, 1912, I, Orig., **65**, 262.

3 Andrewes, F. W., *Lancet*, 1918, **194**, 560.

4 Glynn, J. H., and Starkey, D. H., *J. Bact.*, 1939, **37**, 315.