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A Simple Technique for the Estimation of Radioactive Components of Plasma after the Administration of Radioactive Iodide

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Radioactive components of plasma released from the thyroid gland after the administration of ^{131}I have recently been studied in rats and in cases of thyroid disease by using in conjunction the methods of paper chromatography and radioautography (for a review, see Gross & Pitt-Rivers, 1952). The use of these techniques is time-consuming and not suitable for plasma containing small amounts of radioactivity. Furthermore, we have found losses of radioactive iodide to occur on paper chromatograms, whilst a similar observation has been made in the case of thyroxine by Taurog, Chaikoff & Tong (1950); the technique therefore is not suitable for quantitative work. This communication describes the use of methanol and silver phosphate for the fractionation of plasma components containing radioactive iodine. It has been found that silver phosphate will remove iodide and thyroxine from solution under specified conditions, whilst methanol liberates thyroxine from its state of physiological combination with plasma proteins; at the same time any thyroid protein present is precipitated with the proteins. Suitable application of these two reagents enables comparative estimations of

the above components to be made with rapidity and accuracy. The determinations may easily be carried out on small serial samples of plasma, and enable graphs to be constructed of the relative changes occurring in the various fractions after doses of radioactive iodide have been delivered to the gland. The results obtained in several patients and in rats are described and correlated with chromatographic and radioautographic studies.

EXPERIMENTAL

Precipitation of plasma proteins. (a) Methanol. Plasma (0.5 ml.) was mixed thoroughly with methanol (1.16 ml.) and centrifuged.

(b) $(\text{NH}_4)_2\text{SO}_4$. Plasma (0.25 ml.) was diluted with 27.8% (w/v) $(\text{NH}_4)_2\text{SO}_4$ soln. (4.75 ml.) and a sample (0.2 ml.) taken immediately for counting. The suspension of globulins was then removed by centrifugation and a similar sample of the supernatant taken for counting. This procedure was adopted in order to eliminate the absorption error in counting, which error was due to the high salt content.

Treatment with Ag_3PO_4 . (a) Plasma (0.5 ml.) was diluted with 0.067 M phosphate buffer, pH 7.4 (2.0 ml.) and the solution vigorously shaken for at least 5 min. with Ag_3PO_4 (about 100 mg.).

(b) After removal of plasma proteins with methanol, the supernatant was diluted with an equal volume of phosphate buffer (pH 7.4) and then shaken with Ag_3PO_4 . In each case the excess of Ag_3PO_4 was removed by centrifugation and the residual activity in the supernatant estimated. Since a plasma-protein film absorbs radioactivity, a correction factor for the methanol supernatant counts is necessary. Unless otherwise stated, samples for radioactive assay (0.1 ml.) were air-dried on thin aluminium trays and the radioactivity was estimated with a mica end-window counter. It was found advisable to add carrier iodide and thyroxine (about 100 $\mu\text{g./ml.}$ of plasma) before fractionation procedures were carried out.

Chromatography. Samples (6 $\mu\text{l.}$) of whole plasma were chromatographed on filter paper (Whatman no. 1), using as developing solvent the upper layer of a mixture of either butanol:2*N* acetic acid (1:1) or butanol:dioxan:2*N*- NH_3 (4:1:5, v/v). One-dimensional ascending chromatography was used, and markers of L-thyroxine, 3:5:3'-tri-iodo-L-thyronine, 3:5-di-iodo-L-tyrosine and radioactive iodide were run with each plasma as required. After overnight development, the papers were dried at room temperature, dipped into ninhydrin solution (0.2% in acetone) and hung in a dark cupboard for 2 hr. to allow development of the coloured spots. The paper strips were then radioautographed, using X-ray 'no screen' film, and the positions of the radioactive spots compared with those of the marker substances.

Animal techniques. Inbred Wistar rats of an American strain were used. They were maintained on a paste diet (bread meal, 2 parts; whole-milk powder, 1 part) for 14 days before use. The daily intake of iodine was about 10 $\mu\text{g./150 g.}$ body weight. About 40% of an intraperitoneal dose of carrier-free ^{131}I became localized in the thyroid gland in these experiments. At suitable time intervals after the dose, groups of five animals were killed with ether and exsanguinated via the heart. The blood was diluted with one-quarter volume of isotonic citrate and plasma obtained by sedimentation in the refrigerator. A mean sample from each group was examined by the techniques described above.

Preparation of thyroid extract. The thyroid glands were removed from five adult rats, 6 hr. after the intraperitoneal administration of carrier-free radioactive iodide (150 $\mu\text{C/200 g.}$ body weight). They were ground with powdered glass in a mortar, ice-cold water being added in portions (5 ml. in all). Fresh, cold rat plasma (5 ml.) was then added, the solid removed by centrifuging and the radioactive extract frozen overnight. After thawing, a small amount of insoluble material was removed by centrifugation before use.

RESULTS

The example shown in Table 1 illustrates the application of methanol precipitation and silver phosphate treatment to plasma and thyroid extract from

rats, 6 hr. after a dose of carrier-free radioactive iodide (75 $\mu\text{C/150 g.}$ body weight). In this instance the addition of carrier iodide had no effect on the results. The residual radioactivity in plasma (4%) after direct treatment with silver phosphate is likely to be in protein-bound thyroxine, since its removal was unaffected by adding carrier iodide. The combination of thyroxine and plasma protein is broken by methanol so that all the radioactivity then remains in the methanol solution (Table 1). The saline thyroid extract was diluted with normal

Fig. 1. Direct chromatographic and radioautographic analysis of rat plasma after administration of radioactive iodide, showing protein iodination in (a) when a mixture of butanol and acetic acid was used for development. The iodinated protein appears at the initial spot as can be seen by comparison with chromatogram (b), when butanol/dioxan/ammonia was used for development. The outlined areas indicate the positions of added marker substances developed with ninhydrin.

Table 1. Application of methanol precipitation and silver phosphate treatment to rat plasma and thyroid extract, 6 hr. after administration of radioactive iodide (75 $\mu\text{C/150 g.}$ body weight)

	Percentage activity present as			
	Iodide	'Thyroid protein'	Thyroxine	Globulin
Plasma	96	0	4	0
Thyroid extract in normal rat plasma	3	99	0	98

rat plasma so that the thyroid protein could be precipitated along with the plasma proteins. Virtually all the thyroid activity was protein bound and associated with the globulin fraction of the added plasma. Chromatographic analysis of the thyroid extract confirmed that only a trace of radioactivity was represented by free iodide and thyroxine. We have established that iodide and free thyroxine are quantitatively removed by the silver phosphate treatment described. Free thyroxine has not been detected in any of the plasmas we have examined, so that the amount of radioactivity removed by silver phosphate is a measure of the percentage of iodide present. The accuracy of this measurement could only be diminished by the discovery of relatively significant amounts of free organic iodo-compounds in plasma, capable of reacting in a similar manner with silver phosphate. A similar process of reasoning applies to the treatment of the methanol supernatant from plasma with silver phosphate, which removes iodide and thyroxine, so that the percentage of the latter is given by difference.

We have encountered an iodination process occurring in plasma proteins when butanol/acetic acid was used for developing chromatograms in the presence of radioactive iodide (Fig. 1). This observation emphasizes the need for care in the interpretation of results when acid solvent mixtures are used. Acland (1952) has also commented on the liability to iodination of other plasma components when manipulations are carried out under acid conditions.

Application of methods to patients receiving therapeutic doses of ^{131}I . The results of the application of these techniques to patients at various intervals after the administration of radioactive iodide are shown in Figs. 2A and 2B. Case 1 (iodide-concentrating, secondary carcinoma thyroid; previous surgical thyroidectomy), who received three large doses of radioactive iodide (150, 200 and 380 mc) showed on each occasion an overall picture similar to case 2 (hyperthyroid, cardiac failure; dose, 8 mc). Soon after administration of these doses over 90% of the radioactive material in the plasma of case 1 was protein bound (i.e. not removed by direct

Fig. 2A.

Fig. 2B.

Fig. 2A. Turnover studies of iodide, thyroxine and 'thyroid protein' in the plasma of a patient with an iodine-concentrating metastatic thyroid carcinoma, after surgical thyroidectomy. The patient received three treatment doses of radioactive iodide at intervals of several months. The estimations were made by means of the Ag_3PO_4 extraction and methanol-precipitation techniques described in the text.

Fig. 2B. Turnover studies of iodide, thyroxine and 'thyroid protein' in the plasma of two patients receiving therapeutic doses of radioactive iodide. Case 2 (hyperthyroid) received 8 mc, whilst case 3 (metastasizing thyroid carcinoma) received 190 mc of radioactive iodide to destroy the thyroid gland and possibly also the iodine-concentrating thyroid tumour. No thyroxine was detected in this latter case and practically the entire plasma radioactivity was associated with the 'thyroid protein' fraction.

Fig. 3. Chromatographic and radioautographic analysis of plasma from case 1 (third treatment) reveals the presence of iodide only after 7 hr. In subsequent samples thyroxine was the only detectable soluble component. The tumour was discharging 'thyroid protein' into the plasma in the second sample (28 hr.), which persisted throughout the investigation. The outlined areas indicate the positions of added marker substances developed with ninhydrin.

Fig. 4. Turnover studies of iodide, thyroxine and 'thyroid protein' in the plasma of rats after administration of radioactive iodide. After a dose of $80 \mu\text{c}$ the thyroid gland was only partially damaged so that the plasma curve includes some radioactivity representing thyroxine production by intact thyroid tissue. The dose of $900 \mu\text{c}$ caused total destruction of the gland, so that the plasma-radioactivity curve probably represents the metabolism of 'thyroid protein' absorbed into the blood from the disintegrated tissue.

Table 2. Fractionation of plasma samples after injecting rats with radioactive iodide

(The percentage changes in the radioactivity of rat plasma after treatment with Ag_3PO_4 and methanol, subsequent to the administration of a dose of carrier-free ^{131}I . The amount of 'thyroid protein' appearing in the plasma is related to the degree of damage to the thyroid gland. No measurable amount of 'thyroid protein' appeared in group 1, and the greatest quantity occurred in group 3 when the gland was totally destroyed.)

Group (dose)	Hr. after dose	Residual activity of plasma (percentage of total) after		
		Silver phosphate (loss = iodide)	Methanol precipitation (loss = 'thyroid protein')	Methanol followed by silver phosphate (loss = iodide + thyroxine)
1 ($20 \mu\text{c}$)	6	0	100	5
	54	25	94	5
	121	57	100	—
	167	53	92	—
2 ($80 \mu\text{c}$)	6	4	100	3
	27	47	77	5
	70	53	89	2
	142	48	83	2
	216	67	70	2
3 ($900 \mu\text{c}$)	6	2	100	3
	27	61	48	5
	70	74	79	3
	142	38	80	6
	219	11	60	4

treatment of the plasma with silver phosphate). A considerable percentage of radioactivity remained unprecipitated by methanol and was then removed by silver phosphate, whilst an increasing amount remained associated with the methanol denatured proteins (see below). Typical results of the chromatographic and radioautographic analysis of plasma samples from case 1 (third treatment) are shown in Fig. 3, and show correlation with the analyses described above.

Case 3 (secondary adenocarcinoma thyroid; previous partial surgical thyroidectomy), received radioactive iodide (190 mc) to ablate functioning thyroid and possibly tumour tissue. This subject formed a complete contrast to cases 1 and 2, for with the exception of the first plasma sample which contained 80 % of iodide, over 90 % of the plasma activity was precipitated by methanol. Separation of the globulins with ammonium sulphate suggested that the iodine-containing complex was not homogeneous, for the radioactivity was not confined to this protein fraction (Fig. 2B, case 3).

Fig. 5. Chromatographic analysis (in butanol/dioxan/ammonia) and radioautography of rat plasma 6, 27 and 70 hr. after administration of radioactive iodide ($900\mu\text{C}/150\text{ g. body weight}$). Note the presence of 'thyroid protein' (lowest spot) and the areas corresponding to iodide and thyroxine. To be compared with Figs. 3 and 6. The outlined areas indicate the positions of added marker substances developed with ninhydrin.

Application to rats treated with radioactive iodide. Three groups of normal rats were given doses of ^{131}I of 20, 80 and $900\mu\text{C}/150\text{ g. body weight}$. Fractionation of the plasma samples after various times gave results shown in Table 2 and Fig. 4. After the lowest dose, no methanol-insoluble radioactive material was found in any plasma sample. Administration of 80 and $900\mu\text{C}$ was followed by the appearance of methanol-insoluble radioactive substance within 24 hr., and the amount was greater at the higher dose level. The rate of disappearance of radioactivity from the plasma was much more rapid after $900\mu\text{C}$ (Fig. 4). Examples of combined chromatographic and radioautographic analysis of whole plasma from these animals are shown in Fig. 5, and form an interesting comparison with those from cases 1 and 3 (Figs. 3 and 6).

Fig. 6. Combined chromatographic and radioautographic analysis of plasma from case 3. Apart from the first plasma sample at 7 hr., the radioactivity was confined almost entirely to the 'thyroid protein' spot, the only other component detected being a little iodide. For comparison with Figs. 3 and 5. The outlined areas indicate the positions of added marker substances developed with ninhydrin.

Concurrent microscopical studies of the thyroid glands of rats from these experiments showed some damage when the dose reached $80\mu\text{C}$ and complete disorganization after 24 hr. with $900\mu\text{C}$. A group of animals which had received the latter dose was retained for 4 weeks, when a further dose of the isotope ($500\mu\text{C}$) was given. No radioactivity became localized in the thyroid region, and silver phosphate treatment of plasma samples after various time intervals showed that about 95 % of the circulating activity was iodide. The remainder was protein-bound material insoluble in methanol; this was confirmed by direct chromatographic and radioautographic analysis of these plasmas.

It is worthy of note that we have recognized the presence of di-iodotyrosine in the plasma of rats, 24 hr. after receiving $900\mu\text{C}$ of ^{131}I . So far as we are aware, the presence of this substance in plasma has not previously been reported. Tong, Taurog & Chaikoff (1951) have demonstrated the presence of free di-iodotyrosine in the thyroid gland; its presence in plasma during the destruction of the thyroid gland is therefore not unexpected.

DISCUSSION

These results show that the application of methanol precipitation and silver phosphate treatment to plasma after the administration of ^{131}I readily furnishes quantitative data on the nature of the compounds present. Direct chromatographic analysis of the plasma samples in our series has shown that iodide and thyroxine were the only methanol-soluble components present in significant amounts. These two substances may be distinguished by direct treatment of plasma with silver phosphate in the first instance, which removes iodide; a similar operation on methanol-treated plasma estimates both thyroxine and iodide. Silver thyroxine must be highly insoluble under the conditions specified, for the amino acid is readily removed by silver phosphate even in the minute amounts present in plasma, and in the absence of added carrier thyroxine; it must, however, first be liberated from its protein-bound state by means of methanol. The efficiency of silver phosphate extraction of thyroxine has been confirmed using synthetic radioactive thyroxine added to plasma, when over 95 % was removed from methanol/phosphate buffer. Radioactive tri-iodothyronine behaves in similar fashion, combining firmly with plasma proteins when added to normal plasma. Like thyroxine, it is completely resistant to the action of silver phosphate until the proteins are denatured with methanol. The use of methanol and silver phosphate procedures does not distinguish between thyroxine and tri-iodothyronine but we have only encountered the latter amino acid in one plasma chromatogram

where it formed not more than a few per cent of the total radioactive material present.

Of the three cases described (Figs. 2A, 2B), case 3 presented an entirely different picture from the others, in that throughout the series of samples (subsequent to the first) over 90 % of the radioactive iodine content of the plasma was precipitated by methanol. This material is related to radiation damage of functioning thyroid or tumour tissue (see below) and is now referred to as 'thyroid protein' for convenience. Its stability to methanol is in contrast to the physiological combination of thyroxine with plasma proteins, which is quantitatively broken when the proteins are denatured by the methanol. The soluble residue after methanol treatment in case 3 was iodide, no thyroxine being detected (Fig. 6). The unexpected absence of the latter substance, even from the earlier samples, may imply that the dose of ^{131}I given (190 mc) was sufficient to inhibit the function of the iodine-concentrating tissue present within a few hours, or that it was primarily incapable of producing thyroxine. Reference to Fig. 2 shows that 'thyroid protein' continued to accumulate in the plasma for about 1 week, after which its disposal occupied a similar time. In this case, therefore, we are apparently observing the results of rapid destruction of thyroid substance and/or tumour tissue, commencing within 24 hr. of ingestion of the therapeutic dose. This was apparently complete as judged by a subsequent dose of radioactive iodide (5 mc) some weeks later.

In case 2, the radioactive iodide administered (8 mc) caused transient damage as judged by the proportionate activity of the methanol precipitate (Fig. 2B); no chromatographic evidence is available from this treatment.

Although in case 1 the metastatic tumour concentrated a considerable proportion of the ^{131}I given on each occasion, the amount of active 'thyroid protein' in the plasma only reached about 50 % of the total radioactivity present (Fig. 2A). This maximum was reached very slowly in the first and second treatments in spite of the large doses given, in contrast to the rapidity with which the destructive effects were so manifested in case 3 (within 24 hr.). In the third treatment (380 mc) the peak amount of 'thyroid protein' in the plasma was attained much more rapidly (Fig. 2A), but this proportion was still little more than 50 % of the total radioactivity present. The failure to achieve a near-total conversion of plasma radioactivity into 'thyroid protein' as in case 3, in spite of the retention of a presumably adequate fraction of the large dose of ^{131}I administered, is reflected in the necessity for successive treatments. Another interesting feature in this case was the appearance of iodide in significant amounts after the third dose, whereas it

rapidly disappeared from the plasma in the previous treatments. The predominance of thyroxine in the plasma in its normal state of physiological combination with plasma proteins is not easily interpreted in view of the rapid uptake and destructive potentialities of the radioactive iodide administered. The application of these techniques to further cases is clearly indicated before solutions to these questions may be offered, but the diversity of the results presented here suggests the possible value of future investigations of this kind.

Experiments with rats were undertaken in an attempt to reproduce the results obtained in case 3, and to relate the extent of thyroid damage to the amount of 'thyroid protein' present in plasma. Tong, Taurog & Chaikoff (1952) have already noted the presence of this latter substance by direct chromatography of rat plasma after relatively large doses of radioactive iodide; we have confirmed their chromatographic observations (Fig. 5) and have also applied the methanol and silver phosphate techniques to the plasma samples (Fig. 4). Our data indicates that the onset of thyroid damage occurs more rapidly than was the experience of Tong *et al.* (1952), although the percentage uptake of the dose by the thyroids was the same in both series of experiments. A dose of $900\text{ }\mu\text{C}/150\text{ g.}$ body weight caused only the transient appearance of about 50 % of the plasma maximum radioactivity as 'thyroid protein' (Table 2), although the glands were completely destroyed. This contrasts with case 3, where over 90 % of the plasma radioactivity was precipitable by methanol throughout. In rats, the methanol-soluble material was composed of thyroxine and iodide (Fig. 5) compared with iodide only in case 3 (Fig. 6) and thyroxine only in case 1 (Fig. 3).

Reference to Fig. 4 shows that the total plasma radioactivity fell much more rapidly in rats receiving $900\text{ }\mu\text{C}$ than in those given $80\text{ }\mu\text{C}$. In the former animals, the iodo-protein liberated from the disintegrating thyroids appeared to be quickly metabolized (probably forming the source of the thyroxine and iodide). The radioactive components of the plasma from rats receiving $80\text{ }\mu\text{C}$ will be represented in part by the production of thyroxine by the normal route from undamaged cells, and account for the slower turnover of active material in these animals. After the lowest dose ($20\text{ }\mu\text{C}/150\text{ g.}$ body weight) there was no evidence for the formation of 'thyroid protein', from which it might be inferred that this quantity of radioactive iodide does not cause measurable damage to the gland (Table 2). So far as conclusions may be drawn from these preliminary studies, the metabolic pathway of the 'thyroid protein' in plasma appears to be different in man (case 3) and the rat ($900\text{ }\mu\text{C}$ dose series). These are directly comparable since the radiation dose was sufficient to obliterate all

iodine-concentrating tissue; thus the plasma picture reflects the absorption and metabolism of thyroid iodo-protein from the area of destruction. It may be that iodinated protein from tumour is included in case 3. Where functioning cells apparently continue to exist as in case 1, the metabolism of the iodo-protein is clouded by the continued formation of thyroxine. In rats, our failure to imitate the therapeutic results achieved in case 3 as regards the percentage of methanol-insoluble material in the plasma, is due to its rapid metabolism. Our observation that iodo-protein extracted from rat thyroids is wholly precipitated with the globulins of plasma (Table 1) suggests that the comparable material in the plasma of case 3 was not homogeneous, since it was only partially precipitated with the globulin fraction (Fig. 2B).

Damage to both thyroid tissue and iodine-concentrating tumour is clearly related to the appearance of 'thyroid protein' in the plasma. The question arises whether the quantitative assessment of this substance and thyroxine in the plasma after ^{131}I administration may be of value in the measurement of radiation damage and the effect of treatment. The construction of plasma fractionation curves as described, may enable some correlation to be made between dose and effect and throw light on the biochemistry of iodine fixation in tumour tissue.

SUMMARY

1. The use of silver phosphate treatment and methanol precipitation of plasma proteins is described for the rapid quantitative determination of the relative proportions of thyroxine and iodide in plasma following the administration of radioactive iodide.

2. Methanol precipitation enables the proportion of radioactivity associated with the plasma proteins which is not associated with thyroxine to be measured with facility.

3. The findings in three patients treated with radioactive iodide have been compared with the results of administering destructive doses of radioactive iodide to rats. In all instances the findings were corroborated by application of chromatography and radioautography.

4. The results so far suggest that the metabolism of protein material from the thyroid gland, which appears in plasma after destructive doses of radiation, follows different pathways in man and rat.

5. It is suggested that the construction of curves showing the variation in the relative amounts of these radioactive components of plasma in patients treated with radioactive iodide may prove of value in assessing the effects of treatment. Further light, too, may be thrown on the processes occurring in the thyroid gland and its pathological counterparts.

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The Resolution of Mixtures of C_{16} – C_{24} Normal-chain Fatty Acids by Reversed-phase Partition Chromatography

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Stand oils (fish drying oils) made from the body oil of the pilchard (*Sardina ocellata* Jenyns) are of commercial importance in South Africa and, as part of a general study of these and other polymerized oils (cf. Joubert & Sutton, 1952), the need arose for an analytical method suitable for the separation of mixtures of saturated and unsaturated acids having from 16 to 24 carbon atoms in the chain. For convenience, it was desirable that the method should be applicable on a semi-micro scale.

While the displacement chromatographic method of Holman & Hagdahl (1950) offered a possible approach, it was felt that the elaborate apparatus and technique involved would impair its ready applicability to our problem. For a similar reason an extension of the method of Vandenheuvel & Hayes (1952) seemed unlikely to be suitable, while that of Ramsey & Patterson (1948) was also rejected on the grounds that the higher fatty acids would have partition coefficients too greatly in favour of the less polar phase in the solvent system used.

The method of reversed-phase partition chromatography on benzene-rubber columns using methanol:acetone (3:1, v/v) containing varying amounts of water as mobile phase, has been applied by Boldingh (1950) to the quantitative semi-micro determination of saturated normal fatty acids from C_6 to C_{18} . Chromatography using rubber columns has been claimed by Boekennoogen (1952, not published in detail) to be satisfactory for resolution of mixtures containing saturated acids up to C_{24} , as well as for mixtures of various C_{18} unsaturated acids. Since 'Mealorub' rubber powder was unfortunately not available at the beginning of our work, the choice remained between elution chromatographic methods (Riemenschneider, Herb & Nichols, 1949; White & Brown, 1948; Herb, Witnauer & Riemen-

schneider, 1951), and the reversed-phase partition chromatographic method of Howard & Martin (1950). Recourse was had to the latter, which has been successfully extended to cover the resolution of mixtures of higher saturated fatty acids from 16 to 24 carbon atoms in chain length. Only acids with an even number of carbon atoms have been used for the investigation.

Preliminary experiments have indicated that the method should also be satisfactory for the resolution of unsaturated acids of differing chain length, as well as for acids of the same chain length but differing degrees of unsaturation (cf. Howard & Martin, 1950).

METHODS

The aqueous acetone-medicinal paraffin system of Howard & Martin (1950) was used throughout the investigation. Aqueous acetone concentrations are expressed on a v/v basis (e.g. 70% (v/v) acetone in water).

Preparation of materials

Non-wetting kieselguhr. 'Hyflo Super-cel' (Johns Manville, U.S.A.) was submitted to a process of flotation in water to remove the finer particles. About 5 lb. of kieselguhr were thoroughly mixed with 3 gal. of water and allowed to stand 1 hr., after which the suspended particles were decanted. The process was repeated on the sediment once, and the final product so obtained was dried at 110°. When cool, the material was exposed to the vapour of dimethyldichlorosilane in a partially evacuated desiccator. After 2 hr., the kieselguhr was washed free of acids with absolute methanol and dried at 110°. The product was completely non-wetting when shaken with water.

Medicinal paraffin. Colourless liquid paraffin (sp.gr. 0.880–0.895) was dissolved in pure pentane and percolated through a column of Brockmann grade I alkaline alumina to ensure removal of any acidic material. Evaporation of the pentane gave pure paraffin, used as stationary phase.

Acids. Palmitic, stearic and lignoceric acids were prepared from natural sources by distillation. Arachidic acid

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