Discussion

The results show that enavid in the recommended contraceptive dosage of 5 mg. a day might produce definite changes in the blood-coagulation system in the direction of "hypercoagulability." The number of women treated is small, but the change in coagulability is marked and uniform.

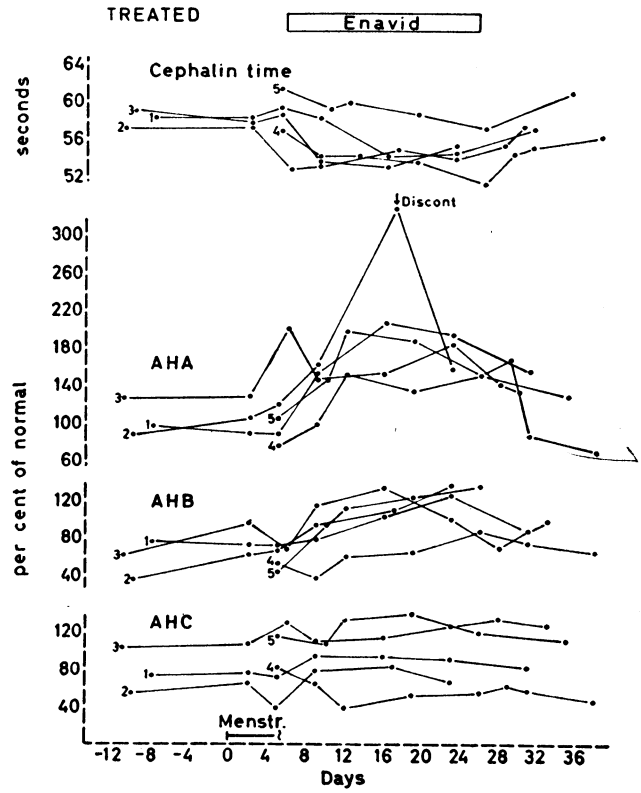

FIG. 3.—Plasma cephalin times and levels of antihaemophilic A, B, and C factors in women on enavid.

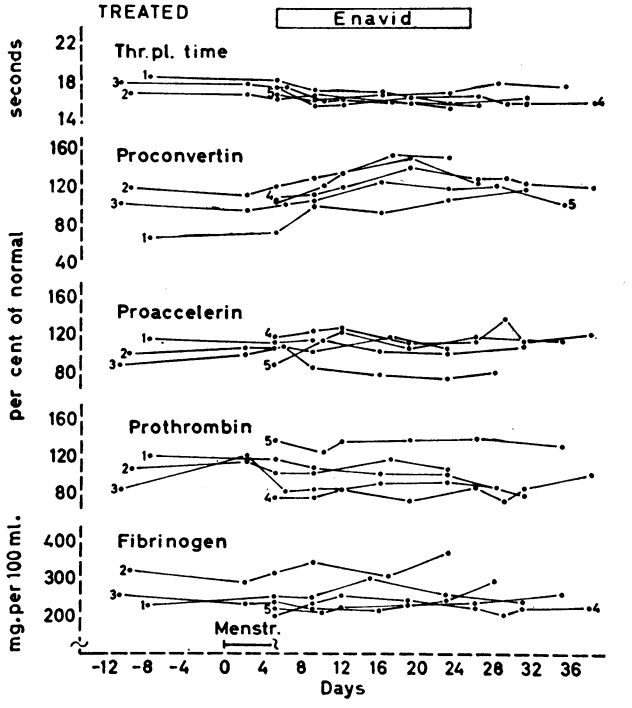

FIG. 4.—Plasma thromboplastin times and levels of factors influencing the extrinsic clotting system in women on enavid.

Summary

Anticonceptive therapy with enavid in five healthy women was followed by increased blood coagulability, as evidenced by shortened plasma cephalin time, marked increase in the activity of the antihaemophilic A factor (factor VIII), and a slight increase in proconvertin (factor VII) activity. No significant changes were observed in blood samples collected at intervals during one menstrual cycle in five non-treated normal women.

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CHLORINATED INSECTICIDE CONTENT OF HUMAN BODY FAT IN SOUTHERN ENGLAND

BY

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Chlorinated insecticides have been used for the control of insect pests of medical and agricultural importance for the past 20 years. Surveys which have been carried out in West Germany and in the United States have demonstrated that one of them (D.D.T.; 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane) is present in the body fat of the general population. No information is available concerning the presence or absence of this material in the body tissues of the population of England. As sensitive modern techniques are available we have carried out a limited survey of the general population of Southern England and analysed samples of human fat for the presence of D.D.T.-derived material. We have also determined the amounts of the active constituent of the insecticide dieldrin in the specimens of human fat; dieldrin is the common name for the product containing not less than 85% of the compound 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,-7,8,8a-octahydro-exo-1,4 - endo - 5,8 - dimethanonaphthalene; this compound is abbreviated as H.E.O.D. in the remainder of this report.

Materials and Methods

Specimens of body fat were obtained from consecutive necropsies carried out at two centres in the South of England; in all, 131 specimens were obtained in the winter of 1961–2. One of the centres was in a semi-rural area, the other was in an urban area. As

there was no special selection of the necropsies from which specimens of fat were taken the specimens may be regarded as a random sample of necropsy fats. The age distribution of the people involved is given in Table I; so far as is known, none had had occupational exposure to insecticides.

TABLE I.—*Age Distribution of 131 Necropsy Subjects*

Age (Years)	No. of Samples					
	Urban		Semi-rural		Total	
	Male	Female	Male	Female	Male	Female
<9	0	0	2	3	2	3
9–19	1	0	1	0	2	0
20–29	0	0	0	0	0	0
30–39	3	3	1	0	4	3
40–49	9	4	3	2	12	6
50–59	13	2	4	2	17	4
60–69	9	8	7	1	16	9
70–79	9	11	5	12	14	23
80–89	3	5	3	4	6	9
90–99	1	0	0	0	1	0

Details of the analytical technique will be published elsewhere. Briefly, the D.D.E.(2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene) and H.E.O.D. present in the unsaponifiable fraction of the fat were determined by means of gas–liquid chromatography using an electron-capture detector. The surveys in the United States (Mattson *et al.*, 1953; Hayes *et al.*, 1958) and West Germany (Maier-Bode, 1959, 1960) have shown that both the ingested D.D.E. and D.D.T. are stored in human fat, the ratio of the stored materials being approximately 45 D.D.T.:55 D.D.E. In the analytical method used in this survey, the stored D.D.T. is converted to D.D.E. by alkaline hydrolysis, and hence the D.D.E. found corresponds to the combined contents of D.D.T. and D.D.E. originally present. No attempt was made to estimate the two compounds individually. The amounts of D.D.E. found have been converted to their equivalent-D.D.T. content—that is, the D.D.T. content was calculated as if all the D.D.E. had been formed by the hydrolysis of D.D.T., by applying the appropriate factor, and it is this equivalent-D.D.T. which is reported in this communication.

Distribution of D.D.T.

The distribution of the equivalent-D.D.T. content of the specimens of fat is shown in Table II. The frequency distribution of this compound is positively skew, and it was found that a normal distribution was obtained from which valid statistical inferences could be drawn if the $\log_{10}$ values were plotted. The sample of 131 specimens of necropsy fat has been subdivided into eight sub-samples corresponding to the two sexes, two living areas, and two causes of death—namely, natural and accidental. The subclassification into natural and accidental death was used, since it appeared possible that natural death may be preceded by a change in the proportion of body fat with a consequent change in the concentration of the insecticide residues. From the means of the transformed results the geometric means of the equivalent-D.D.T. contents of the sub-samples were obtained. These are given in Table III.

TABLE II.—*Equivalent-D.D.T. Content of Specimens of Necropsy Fat*

Equivalent-D.D.T. Content (p.p.m.)	No. of Samples with Equivalent-D.D.T. Content in Specified Range		
	Urban	Semi-rural	Total
0–0·99	9	6	15
1–1·99	20	15	35
2–2·99	21	14	35
3–3·99	15	3	18
4–4·99	10	5	15
5–5·99	4	2	6
6–6·99	0	2	2
7–7·99	1	0	1
8–8·99	0	1	1
9–9·99	0	0	0
10–10·99	0	0	0
11–11·99	0	0	0
12–12·99	0	0	0
13–13·99	1	0	1
14–14·99	0	1	1
15–15·99	0	0	0
16–16·99	0	1	1

TABLE III.—*Mean Content of Equivalent-D.D.T. in Human Fat (Means Derived from Transformed Results)*

Cause of Death	Mean Equivalent-D.D.T. Content (p.p.m.)			
	Urban		Semi-rural	
	Male	Female	Male	Female
Accidental ..	3·3	2·05	2·15	1·7
Natural	2·7	1·7	3·0	1·4

The geometric mean of the equivalent-D.D.T. content of this sample of 131 necropsy fats is 2.21 p.p.m. We conclude that the equivalent-D.D.T. content of the specimens from males, in both the urban and the semi-rural areas, is significantly higher than that of the specimens from females (differences significant at $P=0.01$ and 0.05 for the urban and semi-rural areas respectively). The comparison in the semi-rural area is complicated by the fact that the variance of the equivalent-D.D.T. content of the female fats is significantly greater than that of the male fats. Further, for both areas again, there are no significant differences in the equivalent-D.D.T. contents of the specimens of fat classified according to the two categories of cause of death. Finally, a comparison between the specimens of male fats from the two areas, or of female fats from the two areas, showed no significant differences in the equivalent-D.D.T. content of the specimens within sexes and between areas.

A comparison of the equivalent-D.D.T. content of the fats and the age at death of the persons from whom the specimens were obtained did not show any correlation between age and equivalent-D.D.T. content. As the number of specimens from persons under the age of 10 years is small it would be rash to draw definite conclusions from the present results concerning the comparative amounts of D.D.T. present in the fat of adults and children or babies.

Distribution of Dieldrin

As explained above, dieldrin is the common name for the product containing not less than 85% of the compound H.E.O.D. It was H.E.O.D. that was actually estimated in the specimens of human fat. The distribution of the H.E.O.D. content in the fat of the 131 humans sampled is given in Table IV.

It will be seen that, as in the case of equivalent-D.D.T. content, the distribution is skew and these results have also, therefore, been transformed to $\log_{10}(10^2 . C)$, where C is the concentration of H.E.O.D. in p.p.m. in the specimens of fat. The geometric means of the H.E.O.D. content of the eight sub-samples classified as described above according to cause of death, living area, and sex are given in Table V. A comparison of the transformed results shows that there are no significant differences between the H.E.O.D. contents of male and

female fats in either urban or semi-rural areas. The sub-samples classified according to the categories of cause of death (accidental or natural) are also not significantly different. Furthermore, the H.E.O.D. contents of the specimens of fat from the urban and semi-rural areas do not differ significantly; in other words, the sample of 131 necropsy fats is homogeneous as regards H.E.O.D. content, there being no significant differences between living areas, sex, or causes of death. Finally there is no significant correlation between H.E.O.D. content of the fat and the age of the person.

TABLE IV.—*H.E.O.D. Content of Specimens of Necropsy Fat*

H.E.O.D. Content (p.p.m.)	No. of Samples with H.E.O.D. Content in Specified Range		
	Urban	Semi-rural	Total
0·0–0·09	5	10	15
0·1–0·19	26	6	32
0·2–0·29	19	13	32
0·3–0·39	13	5	18
0·4–0·49	7	8	15
0·5–0·59	3	3	6
0·6–0·69	6	2	8
0·7–0·79	1	1	2
0·8–0·89	0	1	1
0·9–0·99	0	0	0
1·0–1·09	0	0	0
1·1–1·19	1	0	1
1·2–1·29	0	1	1

TABLE V.—*Mean Content of H.E.O.D. in Human Fat (Means Derived from Transformed Results)*

Cause of Death	Mean H.E.O.D. Content (p.p.m.)			
	Urban		Semi-rural	
	Male	Female	Male	Female
Accidental ..	0·28	0·20	0·11	0·18
Natural	0·25	0·19	0·24	0·22

Discussion

The presence of D.D.T.-derived material in this sample of human necropsy fats obtained from two areas of Southern England is not unexpected, as the English diet is similar to that of the Germans and Americans. The equivalent-D.D.T. content of this sample of necropsy fats from the population of Southern England is very similar to that of the sample of fats from the general population of West Germany. It is much less than that in a sample of human fats from the general population of the States of Washington and Georgia in the United States and is very much less than that in certain exposed groups in the United States. The equivalent-D.D.T. contents found in the specimens of human fat in the United States are given in Table VI.

In the United States, where the most extensive surveys have been made, the D.D.T. content of the body fat of persons who had had no occupational exposure to D.D.T. showed no significant increase during the period 1950–6 (Hayes *et al.*, 1958). This indicates that a concentration of D.D.T. in the fat had been reached which was in equilibrium with the dietary intake and the excretionary processes. There is no direct evidence that the equilibrium concentrations in the fat for either D.D.T. or H.E.O.D. have been reached in the population of Southern England; an explicit demonstration of this could be obtained only by analyses of fat specimens from the population over a period of time. However, it is perhaps noteworthy that a highly significant correlation exists between the concentration of equivalent-D.D.T. and that of H.E.O.D. in the specimens of fat of three of the sub-samples classified according to sex and living area. A non-parametric test of association was made, since the distribution functions of the insecticide contents of the specimens of fat appear, as pointed out above, to be non-normal. The Spearman rank correlation coefficients (r_s) for the four sub-samples are given in Table VII.

TABLE VI.—*Content of D.D.T.-derived Material in Body Fat of Human Subjects in Southern England, West Germany, and U.S.A.*

Country	Samples of Fat, No.	Mean Equivalent-D.D.T. Content (p.p.m.)
Southern England	131	2·2
West Germany	60	2·2
U.S.A.:		
General population of States of Georgia and Washington	61	11·7
Human volunteers before trials	51	20·8
Agricultural applicators	30	42·1
Human volunteers after trial—		
6 months at 35 mg. man day	14	238·5
Formulation plant operatives*	1	264·0
Formulation plant operatives†	1	1,134·0
Alaskan natives‡	20	3·0

* Mattson *et al.* (1953). † Hayes *et al.* (1958). ‡ Durham *et al.* (1961).

TABLE VII.—*Spearman Rank Correlation Coefficients (r_s) for the Equivalent D.D.T. and H.E.O.D. Contents of Specimens of Human Fat*

Area	r_s		Probability that Observed r_s May Arise by Chance	
	Male	Female	Male	Female
Urban ..	0·47	0·53	<0·001	<0·005
Semi-rural ..	0·10	0·69	Not significant	<0·001

If it is postulated that the daily intakes per person of D.D.T. and of dieldrin are constant for each of the insecticides and that each of the insecticides has attained independently concentrations in the fat which are in equilibrium with their respective intakes and excretionary processes, the highly significant correlations between D.D.T. and H.E.O.D. contents are easily explicable. If it is not assumed that a dynamic equilibrium concentration has been reached the existence of the observed correlations is more difficult to explain. The non-significant correlation for the semi-rural male is not necessarily in contradiction with the concept of a dynamic equilibrium; it may be the result of variations in the daily intake of either D.D.T. or dieldrin. An examination of the results for this sub-sample indicates that three of the specimens contain small amounts of H.E.O.D. but, relative to the rest of the sub-sample, large amounts of D.D.T.—that is, in this sub-sample the postulate of uniform exposure to D.D.T. may be incorrect.

The dietary intake of the general population of the United States is some 184–202 μg. of D.D.T. per day per person, and it is considered (Hayes *et al.*, 1956) that the equilibrium concentration of the D.D.T. in human body fats is reached in about one year. There is no evidence that the residues of D.D.T.-derived material in the body fat of the general population in the United States are harmful, and we may conclude that the lower amounts found in the general population of Southern England are also innocuous.

The presence of H.E.O.D. in human body fat has not been previously reported, but the very small amounts found are not contrary to expectation in view of the fact that both insecticides are used in agriculture and the presence of D.D.T.-derived material. There are no indications that the quantities found are harmful.

Summary

Analyses of human body fat obtained from 131 necropsies performed in Southern England have shown that D.D.T.-derived material and 1,2,3,4,10,10-hexachloro - 6,7 - epoxy - 1,4,4a,5,6,7,8,8a - octahydro-exo-1,4-endo-5,8-dimethanonaphthalene (H.E.O.D.) are present. The mean content in the fat of the general population is 2.21 p.p.m. equivalent-D.D.T. and 0.21 p.p.m. H.E.O.D.

The results compare favourably, as regards amounts of insecticidal material present, with those found in the general population in West Germany, and more than favourably with those found in the general population of the United States. There are indications that equilibrium levels in the fat have been reached, reflecting the current exposures to these materials.

We express our gratitude to Dr. Francis Camps and Dr. G. B. Forbes for their interest and assistance in this survey.

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A CLINICAL TRIAL OF IRON-FORTIFIED BREAD

BY

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Recent surveys of population groups (Kilpatrick, 1961 ; Kilpatrick and Hardisty, 1961) and reports from general practice (French, 1955 ; Fry, 1961) have revealed high prevalence rates of hypochromic anaemia. This might be reduced and new cases be prevented by an increase in the dietary iron in some universally consumed food. This report describes an experiment designed to assess the utilization of iron, from bread made with flour enriched with a high ferrum redactum content, by a group of adult women over a fairly long period of time.

In great Britain since 1953 all flour with an extraction rate of 80% or less has had iron added to ensure a minimum content of 1.65 mg. of iron per 100 g. of flour (Flour Order, 1953 ; Flour (Composition) Regulations, 1956). Iron, vitamin B_1, and nicotinic acid are added by millers to white flour. Iron may be ferrum redactum (very finely divided metallic iron) or ferric ammonium citrate (Flour (Composition) Regulations, 1956), but only ferrum redactum is known to be used at present (E. M. Laing, personal communication, 1961).

The absorption of iron from bread made with fortified flour was examined by Steinkamp, Dubach, and Moore (1955), who incorporated radio-iron (^{59}Fe) into the iron compounds most commonly used in the U.S.A. A single meal of approximately 100 g. of fortified bread, containing a total of about 3 mg. of iron, was given to 26 male and six female "normal" adult students, and radio-iron was estimated daily in the faeces and later in a blood specimen. Seven of the 32 subjects received ferrum redactum—five absorbed 10% or less, and the other two absorbed 26% and 38%, but both these may have had low iron stores. A vitamin C supplement considerably increased the absorption of each compound, but only one subject receiving ferrum redactum was so treated.

Harrill, Hoene, and Johnston (1957) made iron-balance studies of nine healthy young women with "normal" haemoglobin levels who were maintained on controlled diets for four-week periods. Iron compounds were added to the flour used to bake the bread for the diets so that each subject received just over 7 mg. of iron per day in addition to 5.5 mg. in the basic diet. Three iron compounds were studied in this way, including ferrum redactum, and all were found to be poorly absorbed; the mean absorption of ferrum redactum was 2%.

Widdowson and McCance (1954) found that children who had been undernourished grew and improved equally well when given unlimited quantities of bread, whether made from wholemeal flour, unenriched 70% extraction flour, or 70% extraction flour fortified with iron and B group vitamins to wholemeal standard. All diets consisted of about 75% bread and contained little milk, meat, fish, or cheese. After one year no significant change in haemoglobin or haematocrit level was found in the children on any of the diets.

Method

Preliminary test bakes showed that the content of reduced iron in flour could be increased to about 100 mg./100 g. of flour before changes occurred in the resulting loaf. At this level the crumb colour changed to greyish white although the quality and flavour were unaltered. At 200 mg./100 g. of flour the colour and flavour were altered but not unpleasantly. For the present experiment the bread was made from flour fortified with reduced iron at 60 mg./100 g. of flour, so that the bread was indistinguishable in every way from a normal white loaf.

The subjects studied were 299 adult female patients living in four long-stay units of a mental hospital. The patients were divided into four groups.

Group I (63 patients) received bread made from flour fortified with ferrum redactum for six months. It was assumed that the average consumption of bread per patient was 8 oz. (225 g.) per day, so that approximately 80 mg. of elemental iron per patient per day was supplied in the bread. During the six months of the trial each patient in this group received on average a total of about 14.4 g. of elemental iron.

Group II (61 patients) received the same bread as Group I for six months, but in addition, for the last two months, each patient was also given 50 mg. of ascorbic acid immediately after breakfast and tea, when bread was the main item of the meal. Each patient in this group also received on average a total of about 14.4 g. of elemental iron during the trial.

Group III (67 patients) received tablets of ferrous gluconate which supplied 110 mg. of elemental iron per patient