CCCXIII. IRRADIATION OF FATS.

II. SOME OBSERVATIONS ON METHODS OF ANALYSIS OF OXIDIZED FATS AND ON THE INTERRELATION OF THE RESULTS OBTAINED.

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IN any attempt to study the oxidative effect of irradiation of fats, it is obvious that the methods of analysis employed must play an important part. The tests which may be applied to oxidized fats are characterized by lack of precision as regards both the actual quantitative expression and the knowledge of the chemistry involved. Reference to the paper by Lampitt *et al.* [1935] will show that three methods were selected to illustrate the application of the irradiation technique which was described. These methods had been studied with the object of exact standardization of procedure and an early communication on the subject was confidently predicted. Since the publication of the paper in 1935, however, further investigation has resulted in the application of the Zeiss photometer to the measurement of the Kreis test intensity, and in the development of a modification of the Issoglio determination, which yields figures very different from those usually obtained. The present paper details the results of this work, together with certain references to the aldehyde value [Lea, 1934] and the peroxide value [Lea, 1931].

Many workers have indicated the complexity of the changes which take place when comparatively simple chemical substances are irradiated and it can be assumed that the changes are even more complicated in the case of fats. Much has been written in an endeavour to explain and interpret the results of chemical examination of oxidized fats but such speculations cannot be regarded as being really profitable with the meagre data available. The authors have carried out certain work in an endeavour to correlate the results obtained on a series of fats using the tests mentioned. These results are recorded and certain tentative conclusions have been drawn regarding the interrelationship of the substances determined by the various methods.

EXPERIMENTAL.

1. *The Kreis test.*

The test, as modified by Kerr [1918], is capable of detecting incipient rancidity, and the work of Powick [1923] would appear to prove that the red colour produced in the reaction is due to the presence of epihydrinaldehyde in oxidized fats.

Attempts to express the results quantitatively cannot be considered to be entirely satisfactory, although any one of the proposed methods in the hands of the same investigator is probably sufficiently accurate to give comparative results. Methods of quantitative expression already proposed include:

(1) Matching by means of a colour standard (such as potassium permanganate [Pritzker & Jungkunz, 1927]).

(2) Diluting the fat with a neutral substance (such as light petroleum) until the colour obtained matches a standard colour (such as methyl red at pH 4.8 [Holm & Greenbank, 1923]).

(3) Diluting the fat with light petroleum until it only just produces a positive reaction [Kerr, 1918].

Täufel & Sadler [1934] have recently proposed the removal of the reacting substance from the fat in a stream of nitrogen and its subsequent colorimetric determination, using the Kreis test by comparison with known amounts of epihydrinaldehyde.

The objection to the use of artificial colour standards is the fact that they do not always match the Kreis test colour exactly, and a method has therefore been worked out by the authors for the quantitative expression of the Kreis test by means of the Zeiss photometer, the use of which offers the advantages that the personal element is eliminated from the determination and the use of artificial colour standards is avoided.

(a) *Purification of reagents.* In the Kreis test, purity of the reagents is an important matter if consistently reproducible colour shades are to be obtained. This applies particularly to the dilution method where a small error in the determination of the point at which a positive test is obtained causes an apparently large alteration in the dilution figure. It has therefore been found necessary to purify the methylated ether for use in the Kreis test and also, at times, the light petroleum.¹

Hydrochloric acid should be of A.R. quality and colourless. It is preferably stored in the dark and should be discarded as soon as it develops any colour.

(b) *The dilution method.* A light petroleum solution of the fat of suitable strength is prepared. A series of test-tubes of identical shape and colour is used, and varying amounts of the solution are measured into the tubes, sufficient light petroleum being added to adjust the volumes to 2 ml. The Kreis test is applied and the dilution at which a negative test is first obtained is used as a measure of the Kreis test intensity.

Such a method of determination is obviously subject to a "personal factor", but it is also greatly affected by the colour of the blank solution. If the latter is coloured (owing to unsatisfactory reagents) the point at which a positive test can be detected is materially altered, and the sensitivity of the test is very much reduced. Although such a method is satisfactory for the comparison of fats which are to be examined together, it can hardly be considered as a suitable method of expressing the Kreis test quantitatively.

(c) *Photometric method of quantitative expression of the Kreis test.* In the experiments described herein the technique used has been as follows.

5 ml. of a fat solution in light petroleum are placed in a small separating funnel with 10 ml. concentrated hydrochloric acid and shaken for 2 min. 5 ml. of 0.1 phloroglucinol reagent are then added, and the mixture is shaken for 2 min. The emulsion is run into a 30 ml. centrifuge-tube and whirled (2300 r.p.m.) for 10 min. The volume of the lower acid layer has been found to be 12.5 ml.

¹ Purification of the ether is effected in a manner similar to that advocated by Werner [1933], by shaking 100 ml. with 20 ml. silver nitrate solution (10%) and 20 ml. potassium hydroxide solution (5%) in a separating funnel. After standing some time, with occasional shaking, the lower layer is removed, and, after washing with water, the ether is filtered. The clear ether is dried over fused calcium chloride and filtered. It is then suitable for use in the preparation of the 0.1% solution of phloroglucinol.

When necessary, light petroleum (B.P. 40–60°) is purified by refluxing with concentrated sulphuric acid, removing the acid by washing and drying with fused calcium chloride.

By means of a dry pipette sufficient of the acid layer is transferred to a 2.0 cm. Zeiss photometer cell,¹ and the colour is measured in the instrument against water as a standard, using a suitable filter. A combination of the Wratten mercury green line filter and a didymium filter (No. G 555a Corning Glass Works) has been found to be satisfactory. The limits of the light band transmitted by the combination were sharply defined at 5400 Å. and less sharply at 5550–5600 Å.

A blank determination is made on the reagents using 5 ml. light petroleum instead of the 5 ml. fat solution, and the Kreis test is calculated by use of the formula:

$$\frac{\log B - \log R}{X},$$

where X = wt. of fat in the 5 ml. light petroleum solution, R = photometer reading of the fat solution and B = photometer reading of the "blank" determination.

Table I shows the results obtained by taking two or more different weights of fat in each of a series of seven irradiated fats and demonstrates the accuracy obtainable. Actually, as the result of a large number of such determinations it can be said that the Kreis test intensity figures are reproducible with an error which is not more than 5%. Values obtained by this method have been compared with those obtained by the dilution method, the accuracy of which is

Table I. *Kreis test intensities determined on different weights of butter fat.*

Sample No.	Weight of butter fat in 5 ml. light petroleum solution (g.)	Kreis test intensity
1	0.714	0.30
	0.571	0.30
2	0.453	0.61
	0.272	0.63
3	0.204	1.40
	0.163	1.40
4	0.107	2.28
	0.064	2.32
5	0.077	5.08
	0.062	4.91
6	0.095	5.68
	0.076	5.61
	0.046	5.60
7	0.042	7.32
	0.025	7.56

Table II. *Comparison of Kreis test intensities by the dilution method and the photometric method.*

% oxidized butter fat in fat mixture	Determined Kreis test intensity		Calculated Kreis test intensity for oxidized fat	
	Dilution ratio	Photometer method $\times 10^3$	Dilution ratio	Photometer method $\times 10^3$
100	3100	2090	3100	2090
82.7	1900	1730	2300	2090
66.2	2000	1390	3020	2100
42.1	1000	910	2380	2160
19.7	400	420	2030	2130

¹ In using the glass cell supplied with the instrument it is necessary to smear the cell-cover with "rubber-grease" before it is placed in position. This effectively seals the cell and prevents the exit of acid fumes.

necessarily much less. Table II gives a series of such figures, obtained for different dilutions of oxidized butter fat with fresh butter fat.

The Kreis test intensities by the Zeiss photometer method are shown to be independent of the weight of fat taken for the test, and the greatest error recorded is 2.5 %. The dilution method is shown to be subject to an error of $\pm 20\%$.

2. *The peroxide value.*

The accurate determination of peroxide in fats has been described by Lea [1931]. The following description of the method includes slight manipulative improvements introduced by the authors.

1 g. fat (or less if the peroxide value is high) is weighed into a pyrex boiling-tube, diameter about 20 mm. which is protected from light by means of a brown paper tube. 1 g. powdered A.R. potassium iodide is added and a mixture of 6.5 ml. A.R. chloroform and 13 ml. A.R. acetic acid. A steady stream of nitrogen is passed into the air space above the liquid by means of a narrow glass tube which is widened for about 2 in. at the lower end. The tube passes loosely through a hole in a rubber stopper (which fits into the pyrex tube) and the upper end carries a glass tap. After 3 min. the stream of nitrogen is stopped by means of the tap, and after removal of the brown paper cover, the tube is immediately heated over a small Bunsen flame until the liquid is boiling freely. The tube is then plunged into a boiling water-bath until the boiling liquid has risen to within half an inch of the rubber stopper. The narrow glass tube is then pulled up until its lower, widened portion closes the hole in the stopper. The closed boiling-tube is now shaken vigorously for a few seconds and plunged into ice-cold water for about a minute. The titration is carried out according to Lea's instructions¹ and the number of ml. of *N*/500 sodium thiosulphate required by the iodine liberated from 1 g. fat must be multiplied by 1.6 to give mg. O₂ per 100 g. fat. This method of presenting the results has been adopted in this paper. The greatest precaution should be taken to protect the fat and reagents from the action of direct light.

In the case of fats which have a high peroxide content, it has been found necessary to use less than 1 g. fat, and this has been done when the titration exceeds about 10 ml. The necessity for this precaution is illustrated by the results given in Table III.

Table III. *Peroxide values determined on different weights of fat.*

	Weight of fat taken (g.)	Peroxide value
Ox kidney fat	0.200	63
	0.249	61
	0.507	52
	1.004	46
Butter fat 1	0.050	90
	0.112	94
	0.203	83
	0.512	71
	1.004	59
Butter fat 2	0.049	166
	0.110	162
	0.226	136
	0.551	118
Butter fat 3	0.062	196
	0.102	197
	0.231	160
	0.442	144

¹ The end-point is not good, but with a little practice the titration can be made without difficulty.

3. *The Issoglio value.*

(a) *Kerr's method.* Apart from a few minor points the following instructions for the determination of the Issoglio value follow the method devised by Kerr [1918].

12.5 g. fat and 50 ml. distilled water are placed in a 300 ml. pyrex flask, and heated on a steam-bath (protected from direct light) for 2 hours. At intervals of 15 min., the contents of the flask are swirled. After 2 hours the mixture is filtered through a wet filter-paper (Whatman No. 42 11 cm.) into a 50 ml. graduated flask and, after cooling, the filtrate is made up to 50 ml. with distilled water. 10 ml. of this solution are mixed with 10 ml. of 20 % sulphuric acid in a 300 ml. pyrex flask, and 50 ml. *N*/100 potassium permanganate solution are added. The solution is heated to boiling, the flask being covered by a small watch glass, and the boiling is continued for exactly 5 min. 50 ml. *N*/100 ammonium oxalate solution are added, and the solution is titrated with *N*/100 potassium permanganate at about 60°.

The Issoglio value is calculated from the formula

$$(t_1 - t_2) \times 3.2,$$

where t_1 = No. of ml. *N*/100 potassium permanganate used for oxidizing 10 ml. of filtrate (i.e. extract from 2.5 g. fat), and t_2 is a blank titration obtained in a similar manner on 10 ml. distilled water which has been passed through a wet No. 42 11 cm. filter paper (an average figure is 3.5 ml.).

The Issoglio value is therefore the oxygen equivalent in mg. of the aqueous extract from 100 g. fat.

(b) *Method of boiling with potassium permanganate.* In order to ensure identical treatment during the boiling with potassium permanganate, the following procedure has been adopted. Two Bunsen burners and tripods are used; one burner is used for bringing the solution to the boil, and the other is so adjusted that it is sufficiently hot to keep the solution boiling. A clean glass bead is placed in the liquid in order to prevent overheating. The flask containing the permanganate etc. is placed on the first tripod until it begins to boil, and it is then quickly transferred to the second tripod and allowed to boil for exactly 5 min. The adjustments of the Bunsen burners are not altered during the course of a determination, and in this way the blank and the determination receive exactly the same treatment.

(c) *Treatment for fats of high Issoglio value.* It was discovered, during the course of an investigation on the irradiation of butter-fat, that the Issoglio value did not increase to its expected magnitude for the longer irradiation periods. This was considered to be due to the possibility that, for fats of high Issoglio value, only a part of the water-soluble oxidizable substances was determined under the standard conditions of the test, as given in (a) and (b). This is shown by the following example. An oxidized butter fat having an apparent Issoglio value of 58 was re-examined by making the determination on a mixture of 6.25 g. fat plus 6.25 g. fresh butter fat with an Issoglio value of 2. The result obtained was 38, from which it may be calculated that the true Issoglio value of the oxidized fat was $(38 \times 2) - 2 = 74$.

As a result of a number of determinations it was concluded that, employing the technique in question, this dilution procedure was necessary for fats having an Issoglio value higher than 40, 12.5 g. of the mixture being taken for the determination.

(d) *Progressive oxidation during the period of extraction.* The method, as described above, was used for obtaining the results presented in a previous paper [Lampitt *et al.*, 1935] but subsequently indications were obtained that oxidation of the fat occurred during the extraction period. Experiments were therefore made in which two precautions were introduced to limit the possibility of oxidation occurring during the determination; the addition of a condenser and the production of an inert atmosphere by a nitrogen stream playing on the surface of the reaction mixture. Results of preliminary tests are given in Table IV.

Table IV. *Effect of an inert atmosphere during the Issoglio extraction.*

Sample No.	Issoglio value obtained		
	Original method	Condenser alone	Condenser and nitrogen
1	1.4	1.2	1.4
2	20.5	15.5	9.5
3	60*	46*	24.5
4	103*	73*	26
5	158†	130†	44.5

* Original fat diluted with fresh butter fat in the ratio 8 : 17.

† Original fat diluted with fresh butter fat in the ratio 5 : 20.

It would appear therefore that, when an Issoglio determination is made by the original method, further oxidation of the fat occurs during the 2 hours' extraction period and that, when the fat is already appreciably oxidized, the further oxidation is very considerable. No definite explanation can be offered of the apparently anomalous results obtained with Sample No. 1 which had a low Issoglio value. In the case of fresh butter fat, however, it is probable that in the original method the fat is not oxidized beyond the induction period, and the two methods would not then be expected to give substantially different results.

Further evidence that the higher values obtained by the original method are due to oxidation of the fat is afforded by the results of determinations in which the time of aqueous extraction was varied. The determinations were made on 2.5 g. fat plus 10 g. fresh butter fat (of known Issoglio value), the results being calculated by the method already indicated. From these determinations which are given in Table VII (original method), it appears that the results obtained by the original method have no quantitative significance, except as empirical values, obtained by adherence to a strict procedure.

(e) *Effect of stirring during extraction.* Another aspect of the method which was obviously capable of improvement was the periodic shaking of the fat and water during the 2 hours' extraction, and in this connexion a series of experiments has been carried out in which the periodic shaking was replaced either by gas bubbling or by mechanical stirring. The results are collected in Table V.

From these figures it appears that stirring by nitrogen bubbling is a satisfactory method of ensuring efficient extraction of the fat by the water. The results are definitely higher than when the nitrogen is passed into the flask (and not through the liquid) and they are also slightly higher than those obtained by mechanical stirring. The possibility that oxygen (or some other impurity) in the nitrogen was the cause of these higher results appears to be excluded by the experiments carried out with hydrogen, with washed nitrogen and with nitrogen which had been passed over heated copper. The lower results obtained by

Table V. *Effect of stirring during the Issoglio extraction.*

Sample No.	Nitrogen on surface	Stirring by gas bubbling				Mechanical stirring	
		Nitrogen	Nitrogen water-washed	Nitrogen over heated copper	Hydrogen	Rotary	Up and down
1	10	14	—	—	—	—	—
2	13.5	18.5	—	—	—	—	—
3	—	17	—	—	—	13.5	—
4	—	17.5	—	—	—	16	16
5	—	33	—	—	—	31	—
6	—	18	17.5	—	—	—	—
7	—	19	—	19.5	—	—	—
8	—	20.5	—	—	20	—	—

mechanical stirring are probably due to the fact that the stirrer was small (since it had to be inserted through the neck of the flask) and, for this reason, not sufficiently efficient.

The use of a stream of nitrogen for stirring the fat and water during the 2 hours period is therefore recommended as an improvement in the method. The results so obtained are very different from those furnished by the original method, but the present authors consider that they are more likely to have some chemical significance.¹

In the above experiments the nitrogen stream was adjusted to about 6 l. per hour, but no attempt was made exactly to standardize the speed. This point seemed to be worthy of further examination, and experiments were therefore made in which the rate of flow of the nitrogen was different for determinations which were otherwise identical. The actual speeds were not accurately determined, but they were roughly 6 and 12 l. per hour, and it will be seen from the results given in Table VI that the magnitude of the Issoglio determination was not affected by the rate of flow of the nitrogen.

Table VI. *Effect of speed of nitrogen stream.*

Sample No.	6 l. per hour	12 l. per hour
1	17.5	18
2	34	34
3	41	41.5

In view of the increasing values obtained by the original method with increasing times of aqueous extraction, a similar series of Issoglio determinations was made on the same fat by the new method. The results are given in Table VII.

Table VII. *Effect of increasing period of extraction.*

	1 hour	2 hours	3 hours
Original method	57.5	122	194
New method	36.5	43.5	43.5

It is seen that the figure obtained by the new method after 3 hours is the same as that for 2 hours, and it may be concluded that the extraction is complete in the normal time, viz. 2 hours.

¹ It is obvious that the composition of any products carried over by the nitrogen stream may be of importance. This matter is receiving attention, but it is significant to record that the solution which is obtained by passing the gas through water in a sintered glass bubbler contains substances which are oxidizable by permanganate.

(f) *Recommended procedure.* As an outcome of this investigation it is recommended that the Issoglio value should be determined as follows.

12.5 g. fat and 50 ml. of boiled and cooled distilled water are placed in a 175 ml. flask provided with a single-surface condenser having a ground glass joint. A slow stream of nitrogen is bubbled through the contents of the flask by means of a glass tube passing down the inside of the condenser, and the flask is heated on a boiling water-bath for 2 hours in a location protected from direct light. After this time, the tube is raised so that the nitrogen no longer bubbles through the liquid, and the flask is allowed to remain on the water-bath for a few minutes until the fat layer has separated. The contents of the flask are then filtered through a wet filter-paper (Whatman No. 42 11 cm.) into a 50 ml. graduated flask, and after cooling, the volume of the filtrate is made up to 50 ml. with distilled water. The permanganate equivalent of this solution is determined as previously described.

It is permissible to make determinations on less than 12.5 g. fat, provided that the weight taken is increased to 12.5 g. with fat of known Issoglio value. In this case, the Issoglio value will be given by the formula

$$b + 12.5(c - b)/a,$$

where a = weight of fat taken, b = Issoglio value of fat used for dilution, c = Issoglio value determined on the mixture.

The results of such determinations made on five irradiated butter fats are given in Table VIII. The fresh butter fat used for the dilutions had an Issoglio value of 1.55.

Table VIII. *Issoglio values determined on varying amounts of original fat diluted to 12.5 g. with fresh butter fat.*

Sample No.	Weight of original fat (g.)								
	12.5	8.7	7.5	7.0	6.5	6.25	5.0	2.5	2.0
1	6.0	—	—	6.0	—	—	—	—	—
2	19	—	—	—	19	—	—	—	—
3	32	—	—	—	—	—	30	—	—
4	44	43	44	—	—	—	42	44	—
5	100	—	—	—	—	113	—	—	110

4. *The aldehyde value.*

The determination of "aldehydes" in oxidized fats by formation of the bisulphite compounds was proposed by Lea [1934] and his method has been employed by the present authors without serious modification. Although the end-points of the titrations are somewhat difficult to judge, it is considered that the determination affords an approximate indication of the "aldehyde" content of an oxidized fat. Experience gained during the course of these investigations would indicate that the results are subject to a maximum error of $\pm 15\%$, although the results of duplicate determinations usually agree more satisfactorily. The factors responsible for the erratic results which are sometimes obtained have not been investigated, but the fundamental weakness of titrating sulphite with iodine in bicarbonate solution is a possible explanation. Since the identity of the substances determined is not known, the results have been expressed as (CO) in parts per million, i.e. 1 ml. $N/500$ iodine per g. fat = 28 parts per million of (CO).

5. *Application of the tests described to the examination of irradiated butter fats.*

The method adopted in this phase of the work has been to determine the peroxide value, the Kreis test intensity, the aldehyde value and the Issoglio value on eight samples of the same butter fat which had been oxidized to different extents by irradiation under the mercury quartz lamp. In addition, the first three of these determinations were also made on the fats remaining after the Issoglio extraction had been made (designated "Issoglio fat" in Table IX). In the latter cases, the fat was filtered through a dry fluted No. 41, 9 cm. Whatman filter-paper, without further heating and without undue exposure to light. Table IX gives the results which have been obtained.

Table IX. *Analytical results obtained on irradiated butter fat.*

Sample No.	Issoglio value	Peroxide value			Kreis test intensity			Aldehyde value		
		Original fat (a)	Issoglio fat (b)	Ratio (a-b)/a	Original fat (c)	Issoglio fat (d)	Ratio (c-d)/c	Original fat (e)	Issoglio fat (f)	Ratio (e-f)/e
1	4.0	16	17.5	—	0.22	0.11	0.50	40	35	0.13
2	11.0	39	34	0.13	0.58	0.29	0.50	125	65	0.48
3	17.0	68	55	0.19	1.51	0.72	0.52	160	70	0.56
4	—	74	—	—	1.73	—	—	160	—	—
5	28.5	114	96	0.16	3.31	1.38	0.58	265	115	0.57
6	29.5	119	108	0.09	3.85	1.88	0.51	280	130	0.54
7	38.0	132	108	0.18	3.92	1.67	0.57	290	115	0.60
8	—	144	—	—	4.58	—	—	305	—	—
9	—	158	—	—	6.18	—	—	360	—	—
10	43.5	194	151	0.22	6.12	2.56	0.58	465	215	0.54
11	—	195	—	—	6.82	—	—	400	—	—
12	51.5	210	174	0.17	8.33	3.48	0.58	465	195	0.58

DISCUSSION.

Although no definite conclusions can be drawn from these results, there are several points of interest to which attention may be called.

(a) It would appear to be significant that in the case of eight fats, varying widely in degree of oxidation, the pretreatment of the fat by the modified Issoglio technique results in a reduction of the peroxide value by about 16%, of the Kreis test intensity by about 55% and of the aldehyde value by about 55% (excluding sample No. 1). This would appear to indicate an equilibrium distribution of the various substances between the aqueous and the fat layers during the Issoglio determination. This is not surprising in the case of the last two determinations, but in the case of the peroxide value it is somewhat difficult to understand. If the peroxides were decomposed by water during the 2 hours' extraction, one would expect a greater reduction than one-sixth, and it is difficult to imagine that they are extracted in any strict sense of the word. It is possible, however, that during the extraction period the peroxides react with oxidizable substances (for example, aldehydes) present in the fat, and that their disappearance is to be accounted for in this manner. A further possibility is that two types of peroxide exist in irradiated fats, only one of which is decomposed under the conditions obtained in the Issoglio extraction.

(b) It is perhaps remarkable that the relative reduction in the aldehyde value should in all cases be practically identical with the relative reduction in the Kreis test intensity; in other words, the extraction by water in the modified

Issoglio determination results in the removal of 55% of the bodies giving the Kreis test and 55% of those determined as aldehydes. This would suggest some close relation between the two determinations, but the nature of this relation is not apparent from the results of the determinations on the original fats.

Fig. 1.

Fig. 2.

The Kreis test is associated with epihydrinaldehyde, or with a compound which can give rise to epihydrinaldehyde, and it is of course possible that this substance will be determined and included in the aldehyde value. However, it would appear from Fig. 2 that the magnitude of the Kreis test does not bear any simple relation to the total aldehydes, and it may be concluded, at least tentatively, that the Kreis test substance does not constitute a fixed proportion of the determined aldehydes.

(c) The determinations presented above were carried out with the intention of elucidating the nature of the substances determined in the Issoglio value. It was thought that perhaps the Issoglio value might be a composite determination including definite contributions from the aldehyde value, the peroxide value and the Kreis test, and that it might be possible to discover some simple relations

Fig. 3.

Fig. 4.

between these four determinations. This object has not been definitely achieved, but Fig. 1 shows the relation between the Issoglio value and the reductions in the Kreis test intensities and also a similar curve for the reductions in the aldehyde values.

In the former case, there is obviously no proportionality, but in the case of the aldehyde value, the results are certainly suggestive, especially when consideration is given to the magnitude of the errors inherent in the three determinations involved.

A similarly linear curve is of course also obtained when the Issoglio value is plotted against the original aldehyde value (Fig. 3), since the Issoglio extraction results in the removal of a roughly constant proportion of the aldehydes.

In Fig. 5 the Issoglio value is plotted against the peroxide value and again the curve appears to be a straight line. This is contrary to the authors' previous suggestion [Lampitt *et al.*, 1935], but it should be remembered that the present Issoglio values have been determined by the new method. It will be seen also that a straight line relation appears to hold between the Issoglio values and the reductions in the peroxide values.

Fig. 4 shows that the Kreis test does not vary proportionally with either the peroxide value or the Issoglio value, and this is in accord with observations previously recorded. Similarly there is no proportionality between the Kreis

Fig. 5.

test and the aldehyde value (Fig. 2). Fig. 2 shows also the relation between the aldehyde value and the peroxide value, and the curve appears to be practically a straight line.

A tentative conclusion is that the aldehyde value and the peroxide value increase in rough proportionality and that, since the Issoglio value determines about 55% of the aldehyde value, it is therefore also proportional to the peroxide value.

SUMMARY.

1. A method of evaluating the intensity of the Kreis test colour by the use of the Zeiss photometer has been presented.
2. An improved method of determination of the Issoglio value has been described, the chief modification being the use of a stream of nitrogen for stirring the fat during the period of aqueous extraction.
3. A technique for the determination of peroxides by Lea's method has been described, and it is shown that the weight of fat should not be greater than the amount required to give a titration of about 10 ml. of *N*/500 sodium thiosulphate.
4. Figures are given for determinations of Kreis test intensities, peroxide values and aldehyde values on irradiated fats both before and after the Issoglio determination. The latter has been found to cause a reduction of about 16% in the peroxide value, about 55% in the Kreis test intensity and about 55% in the aldehyde value. These figures were not dependent on the degree of oxidation of the fats.
5. The Issoglio value appears to be proportional to the reduction in the aldehyde value and also to the aldehyde value of the original fat.
6. The aldehyde value and the peroxide value have been found to be roughly proportional. Since the Issoglio value is proportional to the aldehyde value, it is also proportional to the peroxide value.

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