The Comparative Nutritive Value of Mono-, Di-, and Triglycerides[1]

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THE reaction of glycerol with triglycerides in the presence of a suitable catalyst will result in the formation of an equilibrium mixture of mono-, di-, and triglycerides. The product of this reaction, using hydrogenated vegetable oil as the triglyceride source, has been used in hydrogenated vegetable oil shortenings for a number of years. More recently mono-, di-, and triglyceride mixtures made by combining glycerol with lard have been used in shortenings made from animal fats.

The occurrence of mono- and diglycerides is not limited to such commercial preparations. Thus the presence of relatively large quantities of monoglycerides in the pancreas has been demonstrated (11), and monoglycerides have been found in vegetable oils in rather small amounts (12). In addition, the formation of mono- and diglycerides during the digestion of fat has been demonstrated both *in vivo* (8) and *in vitro* (5).

Considerations of the structure of mono- and diglycerides and their natural occurrence would lead one to anticipate that, except for differences in caloric value, they would be nutritionally equivalent to triglycerides. Probably because of the difficulty in preparing the fats in relatively pure form in the quantities needed for nutritional studies, there have been few publications of experimental work to support this concept. Within recent years the development of new procedures has provided tools for the preparation of relatively large quantities of a variety of pure mono-, di-, and triglycerides. Thus molecular distillation (2) of fat allows one to isolate individually the mono- and diglycerides from a mixture of mono-, di-, and triglycerides. Refinements of techniques for the isolation of pure fatty acids and the method of Eckey and Formo (6) for the directed interesterification of glycerides afford procedures for the preparation of mono- or triglycerides of single fatty acid composition.

It was our purpose in this experiment to compare the nutritive values of mono-, di-, and triglycerides of single and mixed fatty acid composition. Since the nutritive values could be influenced by the fatty acid component as well as by the type of glyceride structure, the experiment was planned so as to allow comparisons between compounds having the same fatty acid composition but different glyceride structures. Therefore, for each group of animals fed a mono- and/or diglyceride of a particular fatty acid composition, another group was fed a triglyceride with the same fatty acid composition. In the determining of the nutritional value of these types of compounds another fundamental difference is the higher caloric value of a triglyceride compared to that of a di- or monoglyceride. For this reason, a simple measurement of growth, or even of the efficiency of a certain quantity of food in producing growth, will not suffice. Rather the caloric efficiency—that is the gain in body weight per unit of calories consumed—more truly expresses the nutritional value of the test material. On this basis, the results obtained show mono-, di-, and triglycerides to be nutritionally equivalent.

Procedure

The glycerides studied and the methods used in their preparation are outlined below:

1. Partially hydrogenated 60-40 soybean oil-cottonseed oil
   a) *Triglycerides:* Hydrogenation of the soybean oil-cottonseed oil to an iodine value of 80.
   b) *Mixture of mono-, di-, and triglycerides:* Interesterification at 180°C. of a) with glycerol using sodium hydroxide as a catalyst (7).

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TABLE I

Analyses of Dietary Fats

	Complete melting point	Iodine value	Saponification no.	Monoglyceride[b]	Fatty acid composition[a]			Caloric value
					Saturated	Oleic	Linoleic	
	°C.			%	%	%	%	*Cal./g.*
Soybean oil/cottonseed oil (partially hydrogenated)								
Triglyceride	31.5	80.5	192	....	16	75	9	9.4
Mono + Di + Triglyceride	39.0	73.8	175	24.9	15	76	9	9.0
Diglyceride	44.0	75.0	182	2.9	16	76	8	9.2
Monoglyceride	53.5	65.5	160	97.7	16	76	8	8.5
Olein								
Triglyceride	3.2	83.8	189	0.2	1	99	0	9.4
Monoglyceride	32.2	72.3	235	95.0	0	98	2	8.6
Stearin								
Triglyceride	72.2	0.0	188	....	....	....	....	9.6
Monoglyceride	76.9	0.9	157	97.5	....	....	....	9.0
Laurin								
Triglyceride	46.5	0.0	261	....	....	....	....	9.0
Monoglyceride	61.0	0.0	205	98.1	....	....	....	7.9
Coconut oil								
Triglyceride	25.4	9.4	255	....	91	7	2	9.0
Mono + Di + Triglyceride	31.8	8.9	155	20.2	89	10	1	8.6
Soybean oil[c]	....	134.7	191	....	18	15	67	9.4

[a] Based on iodine and thiocyanogen values. [b] Method of Handschumaker and Linteris (9). [c] Refined, bleached, and deodorized soybean oil used at a level of 1% of each diet. See Table II.

c) *Monoglycerides:* Molecular distillation of b) in a 5-inch centrifugal still (2).
d) *Diglycerides:* Similarly prepared by molecular distillation of b).

2. Olein
   a) *Triolein:* Interesterification of glycerol with purified methyl oleate (14) using sodium glyceroxide as a catalyst.
   b) *Monoolein:* Molecular distillation of superglycerinated olive oil followed by fractional crystallization at low temperature from hexane and ethyl alcohol.
3. Stearin
   a) *Tristearin:* Recrystallization from hexane of hydrogenated linseed oil (iodine value 1.0).
   b) *Monostearin:* A mixture of glycerol, unhydrogenated, and hydrogenated (iodine value 1.0) linseed oil was rearranged at random. This product was then subjected to directed interesterification (6). The monostearin was isolated by recrystallization from hexane and ethyl alcohol.
4. Laurin
   a) *Trilaurin:* Esterification of glycerol with purified lauric acid, using *p*-toluene sulfonic acid as the catalyst followed by recrystallization from hexane and ethyl alcohol.
   b) *Monolaurin:* High temperature randomization of lauric acid with glycerol, followed by crystallization from hexane and ethyl alcohol.
5. Coconut Oil
   a) *Triglyceride:* Refined, bleached, and deodorized coconut oil.
   b) *Mixture of mono-, di-, and triglycerides:* Interesterification of a) with glycerol (7).

The analytical values obtained on these fats are given in Table I.

One hundred twenty weanling, male, Sprague-Dawley rats were distributed into 12 equal groups on the basis of litter and body weight. The animals were housed in individual cages and offered their appropriate diet and water *ad libitum.* The composition of the diet used is shown in Table II. It will be noted that the test fats were added on an equal weight basis so as to constitute 25% of the diet.

The animals were weighed at weekly intervals, and throughout the experiment record was kept of the quantity of food consumed. At the end of the experiment, 10 weeks, the animals were sacrificed. Autopsies were performed on all animals and sections of various tissues were taken for histological examination. At the time of autopsy the perirenal fat was removed and repeatedly extracted at room temperature with ethyl alcohol followed by diethyl ether. The fats so isolated were partially characterized by analysis. The Official Methods of the American Oil Chemists' Society were used for the analyses of the

TABLE II

Composition of Experimental Diets [a]

Casein (Labco, Vitamin Free)	27.0
Sucrose	36.0
Salt mix, Hubbell (10)	4.0
Fortified sucrose [b]	5.0
Liver powder (Wilson 1:20)	1.0
Celluflour	1.0
Soybean oil [c]	1.0
Test fat	25.0

[a] Caloric value of diet minus test fat, 290 calories/75 grams.

[b] Purified vitamins added (mgm./100 gm. diet)

Thiamine HCl	0.4
Niacin	2.0
Choline HCl	300.0
Inositol	200.0
Biotin	0.03
Menadione	0.3
Riboflavin	0.5
Pyridoxine HCl	300.0
Calcium pantothenate	2.0
Folic acid	0.25
p-Amino benzoic acid	10.0
Ascorbic acid	10.0

[c] Purified vitamins added so each 100 grams of diet contained: 1250 USP units of vitamin A, 125 units of vitamin D, and 10 mgm. of α-tocopherol.

TABLE III

Average Cumulative Values and Standard Error of the Means [a] for Gain in Body Weight, Food Consumption, and Caloric Efficiency

	Third Week			Sixth Week			Tenth Week		
	Body weight gain	Food consumed	Caloric efficiency [b]	Body weight gain	Food consumed	Caloric efficiency [b]	Body weight gain	Food consumed	Caloric efficiency [b]
	g.	*g.*		*g.*	*g.*		*g.*	*g.*	
Soybean oil/cottonseed oil (partially hydrogenated) Triglyceride	132.9 ±2.7	225.7 ±4.7	11.1 ±0.15	240.0 ±4.9	538.1 ±12.6	8.51 ±0.11	305.0 ±7.7	938.1 ±25.0	6.20 ±0.11
Mono + Di + Tri	133.3 ±3.4	227.0 ±5.4	11.3 ±0.12	242.0 ±6.7	542.8 ±12.4	8.65 ±0.09	314.3 ±8.8	957.3 ±21.7	6.37 ±0.08
Diglyceride	129.3 ±5.9	223.3 ±7.71	11.1 ±0.19	236.2 ±7.4	534.7 ±14.7	8.49 ±0.05	311.8 ±14.8	930.8 ±31.9	6.41 ±0.12
Monoglyceride	122.7 ±3.1	212.8 ±3.2	11.5 ±0.16	207.2 ±9.4	493.6 ±12.6	8.34 ±0.20	275.8 ±9.1	866.5 ±20.3	6.30 ±0.08
Olein Triglyceride	115.9 ±2.7	200.4 ±5.0	10.9 ±0.15	220.1 ±3.9	491.8 ±8.0	8.52 ±0.09	287.9 ±6.1	867.2 ±15.4	6.32 ±0.07
Monoglyceride	112.5 ±2.8	198.1 ±3.6	11.1 ±0.20	210.6 ±5.0	475.7 ±8.4	8.77 ±0.13	270.0 ±4.9	850.0 ±11.7	6.29 ±0.08
Stearin Triglyceride	108.6 ±1.5	319.4 ±2.6	6.40 ±0.10	206.0 ±1.2	773.7 ±7.3	5.02 ±0.09	274.0 ±4.4	1417.0 ±15.2	3.64 ±0.05
Monoglyceride	95.5 ±1.6	281.9 ±3.6	6.58 ±0.10	202.6 ±2.8	716.6 ±10.0	5.50 ±0.06	271.0 ±3.1	1314.0 ±14.8	4.01 ±0.06
Laurin Triglyceride	113.2 ±3.3	225.3 ±4.7	9.75 ±0.18	203.9 ±7.6	518.3 ±10.2	7.60 ±0.17	276.6 ±10.1	931.7 ±19.3	5.77 ±0.09
Monoglyceride	75.0 ±2.6	171.1 ±5.4	8.99 ±0.26	155.4 ±3.3	418.7 ±10.5	7.61 ±0.12	214.8 ±5.0	779.9 ±18.4	5.65 ±0.06
Coconut oil Triglyceride	140.8 ±2.2	239.1 ±4.7	11.4 ±0.11	255.5 ±4.4	565.4 ±10.6	8.90 ±0.14	333.8 ±6.4	991.9 ±18.6	6.60 ±0.08
Mono + Di + Tri	130.0 ±3.0	222.5 ±4.4	11.6 ±0.11	237.1 ±5.1	524.8 ±6.8	8.83 ±0.07	304.9 ±7.3	923.9 ±12.9	6.54 ±0.11

[a] Standard error of the mean calculated by the formula $\sqrt{\frac{\Sigma(\bar{x}-\bar{x})^2}{n(n-1)}}$ where x is the individual value, $\bar{x}$ is the group mean, and n is the number of observations.

[b] Body weight gain/calories consumed × 100 = caloric efficiency.

perirenal and dietary fats. Monoglycerides were determined by the method of Handschumaker and Linteris (9).

Results

Because of the volume of data obtained on body weight gain, food consumption, and caloric efficiency, only the values for the end of the third, sixth, and tenth weeks are reported. The results for the intervening weeks parallel those reported here. These data are summarized in Table III. Values at the end of the tenth week are shown graphically in Figures 1-3.

Gain in Body Weight. The values for gain in body weight (Table III, Figure 1) show that, in general, where the glycerides consisted of pure fatty acids—that is oleic, stearic, or lauric—the growth rate was inferior to that of the animals fed glycerides containing mixed fatty acids. Thus the largest gain of all was made by the animals fed coconut oil, followed next by those fed the soybean oil-cottonseed oil mixture. Comparisons of the growth rates on the basis of glyceride structure within a fatty acid group show:

a) In the soybean oil/cottonseed oil group, all groups gained essentially the same, except the monoglyceride-fed animals which grew at a slightly lower rate.
b) In the olein and stearin groups the differences between the animals fed mono- or triglyceride are not significant.

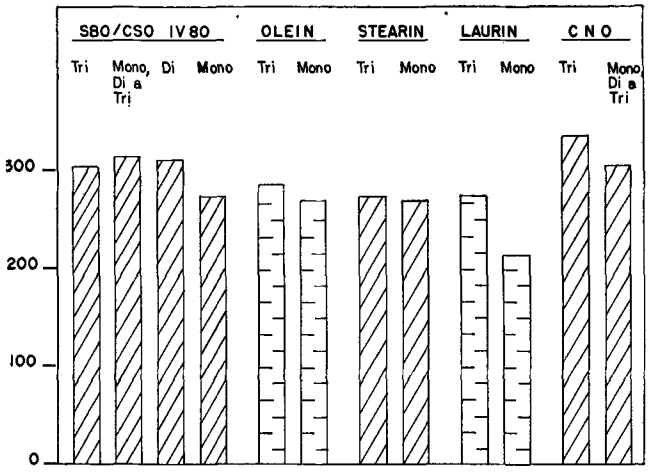

FIG. 1. Average gain in body weight after 10 weeks on the experimental diets.

c) The growth of the monolaurin-fed group was markedly inferior to that of the trilaurin-fed group.
d) The animals fed superglycerinated coconut oil grew at a rate slightly less than that of the coconut oil-fed animals.

Food Consumption. The average values for food consumption (Table III, Figure 2) show that there were great differences in the quantity of the diets eaten. The following points are worthy of note:

a) The relatively large quantities of food consumed by the stearin-fed animals.
b) The smaller food consumption by the animals fed the monoglycerides of soybean oil/cottonseed oil, mono- or triolein, and particularly monolaurin.
c) The higher consumption of the coconut oil diet relative to all other diets except the stearins.

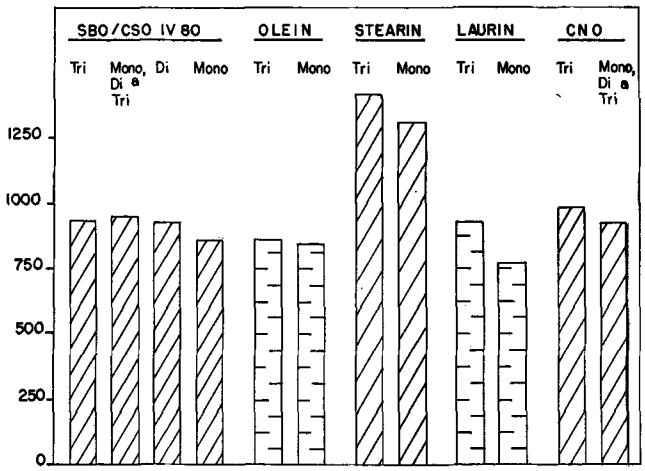

FIG. 2. Average total food consumption for 10 weeks on the experimental diets.

The values for food consumption explain, in part at least, the differences observed for gain in body weight. In almost every instance where there was inferior growth, this was accompanied, or probably one should say preceded, by a smaller food consumption. The outstanding exceptions to this observation are the animals fed mono- or tristearin.

Caloric Efficiency. One further point needs to be brought out in relation to the growth of these animals. Under the experimental conditions employed the test materials were substituted in the diet on an

TABLE IV

Analyses of Perirenal Fats

	Iodine value	Saponification no.	Monoglyceride	Fatty acid composition[a]		
				Saturated	Oleic	Linoleic
Soybean oil/cottonseed oil (partially hydrogenated)			%	%	%	%
Triglyceride	84	194	0.34	13	76	11
Mono + Di + Triglyceride	82	187	0.41	16	73	11
Diglyceride	82	191	0.54	16	74	10
Monoglyceride	83	190	0.05	13	77	10
Olein						
Triglyceride	79	192	0.01	7	93	0
Monoglyceride	82	193	0.08	9	86	5
Stearin						
Triglyceride	59	195	0.55	36	59	5
Monoglyceride	56	196	0.39	38	58	4
Laurin						
Triglyceride	34	214	0.11	63	33	4
Monoglyceride	37	219	0.29	61	36	3
Coconut oil						
Triglyceride	36	224	0.08	63	31	6
Mono + Di + Triglyceride	39	220	0.14	60	35	5

[a] Based on iodine and thiocyanogen values.

equal weight basis. However, as can be seen in Table I, the caloric values of these fats are not the same. Thus the caloric value of a monoglyceride is considerably less than that of its corresponding triglyceride. These values for the experimental fats were not determined biologically but were calculated. To arrive at those for the soybean oil-cottonseed oil series and the coconut oil series, typical fatty acid distributions compatible with the iodine and thiocyanogen values of these fats were used. Thus the caloric values for the fats of mixed fatty acid composition may be slightly in error.

Because of differences in the caloric value of the diets and differences in the quantity of food consumed, caloric efficiency, i.e., the gain in body weight per 100 calories of diet eaten, more truly expresses the nutritional value of the test substances. These results are shown in Table III and Figure 3. It is quite apparent that the animals fed the stearins and laurins showed an impaired caloric efficiency. Between the remaining test fats no real differences are to be found except for the slightly higher caloric efficiencies shown by the coconut oil series.

This tendency for the coconut oil series to show a slight superiority could well be due to the errors inherent in calculating the caloric value of a natural fat. Comparisons of caloric efficiencies between glycerides characterized by the same fatty acids show that there is essentially no difference which can be attributed to glyceride structure with the exception that monostearin was slightly better than tristearin.

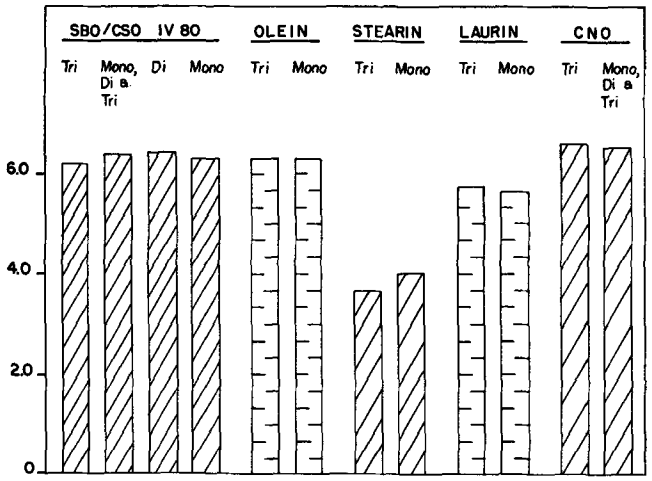

FIG. 3. Average cumulative values for caloric efficiency during 10 weeks on the experimental diets.

Autopsies and Histopathologies. Throughout the experimental period all animals had a normal appearance. Autopsies performed at the end of the experiment and histological examination of the liver, kidney, spleen, lung, heart, stomach, and small and large intestines revealed no abnormalities attributable to the consumption of any of the test materials.

Character of Body Fat. The analytical values obtained on the perirenal fats of the animals are shown in Table IV. The low iodine values and high saponification numbers of the laurin and coconut oil groups reflects the type of fat fed. Thus there was deposition of the dietary short chain fatty acids. Similarly the low iodine values of the body fats of the stearin-fed animals reflect this characteristic of the dietary fats. The values for the composition of the body fat, as determined by iodine and thiocyanogen values, confirms this tendency for body fat to be influenced by the nature of the dietary fat. However such a sequence does not occur with respect to the type of glyceride structure fed. Thus, regardless of whether mono-, di-, or triglycerides were fed, the body fat deposited was essentially the same. The small amount of monoglycerides found represent either a normal level which is characteristic of the body fat or may be those formed as the result of partial hydrolysis of triglycerides during the isolation of the body fat.

Discussion

The results obtained in this experiment show that, except for differences in caloric value, mono- and diglycerides are nutritionally equivalent to triglycerides of corresponding fatty acid composition. That such should be the case is not surprising since mono- and diglycerides are obligatory intermediates in the hydrolysis of triglycerides. Thus the hydrolysis which occurs in the process of digestion of triglycerides is a series of stepwise reactions yielding first diglycerides, then monoglycerides, and finally glycerol with fatty acid released at each stage. Dietary mono- and diglycerides would then be treated by the body as partial digestion products of triglycerides. This concept is supported by the observation that the perirenal fats are identical regardless of whether a mono-, a di-, or a triglyceride is fed.

This conclusion as to the equivalence of mono-, di-, and triglycerides is in agreement with reports by others. Thus Braun and Shrewsbury (3) found monostearin and monolinolein to be nutritionally equivalent to lard in producing growth in rats. Ames *et al.* (1) have recently reported nutritional studies on monoglycerides isolated by molecular distillation. They found no difference in the growth, reproduction, or lactation performance of rats fed mono- or triglycerides of cottonseed oil.

The differences observed in this experiment in the quantities of diet consumed are not at all surprising, considering the range of physical properties included in this series of fats. To mention only one of these, the melting points varied from a low of 3.2°C. in the case of triolein to a high of 76.9°C. in the case of monostearin. In the studies reported by Scott (13) on the self-selection of diets containing various fats marked differences in food consumption were also observed. These differences he attributed to the animal's subjective response to the diets rather than to any inherent nutritional factors. A similar interpretation would seem to apply to the food consumption values reported here.

The inferior caloric efficiencies shown by the diets containing the mono- and triglycerides of stearic and lauric acid are probably due to poor absorption of these particular fats. Thus Cheng *et al.* (4) have reported utilization values of approximately 20% for mono- and tristearin and 70% for trilaurin. Such poor utilization of a material which constitutes from 40 to 45% of the total calories in the diet could easily account for the poor caloric efficiencies shown by these diets. The large quantities of food eaten by the animals fed mono- and tristearin is probably due to these animals having to consume far more food than

the others in order to obtain even comparable quantities of calories which are actually absorbed.

Summary

Weanling rats were fed diets containing various pure mono-, di-, or triglycerides at a 25% level for 10 weeks. The following results were obtained:

a) Mono-, di-, and triglycerides of corresponding fatty acid composition were of equivalent caloric efficiency.
b) The caloric efficiencies of the mono- and triglycerides of pure lauric or stearic acid were found to be low. This may have been due wholly or partially to poor absorption.
c) Autopsies and histological examination of the tissues of the animals revealed no abnormalities attributable to the consumption of any of these fats. Appearance of all animals was normal throughout the experiment.
d) The body fat of the animals was the same regardless of the type of glyceride structure fed. However the type of body fat deposited reflected, in part, the fatty acid component of the dietary glyceride.

From these results it is concluded that, except for differences in caloric value, mono-, di-, and triglycerides of corresponding fatty acid composition, are nutritionally equivalent.

Acknowledgments

We wish to thank Frank Cleveland, M.D., of Kettering Laboratory, of the University of Cincinnati for carrying out the autopsies and histological examinations.

Addendum

Since carrying out the work reported here, we have investigated further the method for determining monoglycerides. In confirmation of the work of Kummerow and Daubert (15), we have applied the method of Handschumaker and Linteris to fats which should be monoglyceride free and have obtained apparent monoglyceride values of up to 0.5%. Thus the analytical values for the monoglyceride content of the perirenal fats reported in this paper are within the experimental error of the method.

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Controlling the Halogen Ratio in Hanus or Wijs Solutions

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IT is well known that the iodine value, whether determined by the Hanus or Wijs methods, is affected by the ratio of the halogen content, iodine to bromine or iodine to chlorine, in Hanus or Wijs solutions. To obtain accurate results it is recommended that the relation I/Br or I/Cl be not higher than unity.

The conventional procedure for determining this ratio is as described in the A.O.C.S. Official Method Cd 1-25, in which the reagent is titrated before and after chlorination.

A method which is possibly more convenient than checking the ratio during the preparation of the solutions has been developed at the Institute. It consists of two titrations: a) iodine titration in the presence of the other halogen by the modified Winkler's method; b) iodometric titration of the total halogen content in the same solution in the usual way.

Procedure

First Titration. Iodine content, buret reading = A ml.

a) Pour about 150 ml. of saturated chlorine water into a 500-ml. Erlenmeyer flask and add some glass beads.

b) Pipet 5 ml. of the Hanus or Wijs solution under analysis into the flask containing saturated chlorine water. Shake and heat to boiling.

c) Boil briskly for 10 minutes, cool and add about 30 ml. of 2% sulfuric acid and about 15 ml. of a 15% KI solution.

d) Mix well and titrate immediately, with 0.1 *N* thiosulfate solution, using starch indicator solution.

Second Titration. Total halogen, buret reading = B ml.

a) Pour about 150 ml. of recently boiled distilled water into a clean and dry Erlenmeyer flask, add about 15 ml. of 15% KI solution, and pipet 20 ml. of the same Hanus or Wijs solution into the flask.

b) Mix well and titrate immediately with 0.1 *N* $Na_2S_2O_3$ solution.

Calculate the halogen ratio by the formula:

$$R = \frac{2A}{3B - 2A}$$

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