Summary. When MER-25 is administered orally to female rats on day of mating and continued throughout the normal period of oviducal passage of ova, implantation does not occur. Restricting treatment period to only one day post-coitus can also be effective in preventing nidation. Treated females enter a post-copulatory period of pseudo-pregnancy, then resume normal estrous cycles. The anti-fertility effects of MER-25 appear to be exerted prior to exit of ova from the oviducts. The compound does not act by preventing decidua development, nor by interfering with post-copulatory maintenance of luteal body function. Histologic evidence indicates that ova of females treated with MER-25 undergo degeneration after extrusion of 2nd polar body.

1. Nelson, W. O., Steinberger, E., *Anat. Rec.*, 1953, v115, 352.

2. Craig, A. W., Fox, B. W., Jackson, H., *Nature*, 1958, v181, 353.

3. Pincus, G., Chang, M. C., Hafez, E. S. E., Zarrow, M. X., Merrill, A., *Science*, 1956, v124, 890.

4. Shelesnyak, M. C., *Am. J. Physiol.*, 1955, v180, 47.

5. Thiersch, J. B., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 33.

6. Smith, M. G., *Bull. J. Hopkins Hosp.*, 1926, v39, 203.

7. Hain, A. M., *Quart. J. Exp. Physiol.*, 1935, v42, 131.

8. Huggett, A. St. G., Pritchard, J. J., *Proc. Roy. Soc. Med.*, 1945, v38, 261.

9. Segal, S. J., Nelson, W. O., *Anat. Rec.*, 1958, in press.

10. Segal, S. J., Nelson, W. O., Boccabella, A., *Fed. Proc.*, 1958, v17, 145.

11. Lerner, L. J., *ibid.*, 1958, v17, 388.

12. Thompson, C. R., Lerner, L. J., *Endocrin.*, 1958,

Received May 2, 1958. P.S.E.B.M., 1958, v98.

Serum Cholesterol in Man and the Unsaponifiable Fraction of Corn Oil in the Diet.*† (24069)

FRANCISCO GRANDE, JOSEPH T. ANDERSON AND ANCEL KEYS

Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis and Hastings State Hospital, Hastings, Minn.

A decrease in serum cholesterol level has been found when corn oil is used in the diet, in substantial amounts in man(1-6) and in the cholesterol-fed chick(7). It has been suggested that much of this effect is due to something besides the fatty acid glycerides in corn oil(8). For these reasons we excluded comparisons involving corn oil in our recent analysis of a large series of fat-feeding experiments(9,10). This analysis indicated that with many fats (butterfat, olive oil, cottonseed oil, sunflower seed oil, sardine oil and mixed fats of ordinary foods), a change in fat content of the human diet results in a change in serum cholesterol level which is predictable from a regression equation, cholesterol rising with increasing saturated and falling with increasing poly-unsaturated fats in the diet: Δ Chol. $= 2.74\ (S_2 - S_1) - 1.31\ (P_2 - P_1)$, (Equation 1) where S and P are percentages of total calories provided by glycerides of saturated and of poly-unsaturated fatty acids, respectively, and the subscripts refer to first and second diets. When data from dietary changes involving corn oil were tested with this equation, the results tended to show that corn oil produced slightly lower serum cholesterol values than predicted. This would conform to the suggestion that corn oil may contain a special cholesterol-lowering substance not accounted for as fatty acid glyceride, presumably in the unsaponifiable frac-

* The studies were aided by research grants from Nat. Dairy Council, Chicago, and Minnesota Heart Assn.

† We are grateful to Drs. R. A. Reiners and G. E. Inglett of Corn Products Refining Co., Argo, Ill., for preparation and analysis of the corn oil unsaponifiable fraction and to Drs. L. W. Beck, P. W. Ifland and F. H. Mattson, Research Division of Miami Valley Laboratories, Procter and Gamble Co., for analyses of the oils used.

tion. Accordingly, it was decided to examine this question further. In the first place, a more critical analysis was made of data from experiments reported previously. Three switchback dietary comparisons in which any time trends are automatically cancelled out were selected from Exp. H and J(11) and are separately analyzed below. Secondly, a new experiment (Exp. N) with this switchback arrangement was performed comparing safflower and corn oils. Finally, a similar experiment, Exp. O, was performed to test the effect of a concentrate of unsaponifiable matter in corn oil. All of these results are reported below.

Methods. Physically healthy schizophrenic male subjects were selected and controlled as in earlier experiments(11). Their ages were from 39 to 66 yrs. and relative body weights from 72 to 119% of standard for their age and height(12). Out of 98 subjects only 5 suffered acute illnesses or had body weight changes of 2 kg in a dietary period or 3 kg in an entire experiment. The data of these 5 men were excluded. A basic diet low in fat (about 40 g per day), as previously described (11), was devised so that calorie equilibrium would be achieved when 100 g of experimental fat were added to the daily ration by incorporating it in the basic diet just before serving by adding it to the cooked hot dishes. Plate rejections were recorded and the diet was adjusted as needed to keep body weight constant as in previous experiments(11). The experimental fats used in the 3 comparisons from Exp. H and J have been reported elsewhere(10,11). In Exp. N commercial samples of corn oil (Mazola) and safflower oil (Pacific Vegetable Oil Co.) were used. Their fatty acid composition was: corn oil—15, 26 and 59% saturated, mono-ene and polyene, respectively; safflower oil—12, 10 and 78% of these fatty acids. In Exp. O the plan was to measure the effect on serum cholesterol of addition to and removal from the diet of the unsaponifiable fraction of corn oil in the presence of a mixture of cottonseed and safflower oil (100 g daily) approximating corn oil in proportions of saturated and linoleic acids. Compositions of oils and the final mixture (imitation corn oil) are given in Table I.

TABLE I. Distribution of Glycerides of Saturated (S), Mono-ene (M) and Polyunsaturated (P) Fatty Acids in the Oils Used in Experiment O.

Oil	Amount in g			
	Total	S	M	P
Cottonseed	56	13.4	12.9	29.7
Safflower	44	5.3	4.4	34.3
Mixture fed*	100	18.7	17.3	64.0

* Imitation corn oil.

Unsaponifiable fraction of corn oil. The unsaponifiable fraction in Exp. "O" was prepared from corn oil having an unsaponifiable content of 1.6%. Eighteen gallons of corn oil were saponified by refluxing one hour with 5.5 times its weight of methanol and 3 times the theoretical amount of NaOH. The pH was then brought to 9-9.5 with HCl, and the mixture was slightly cooled and dried. The dry flakes were washed in 300 gallons of acetone, the mixture being agitated mildly and extracted overnight at room temperature. Extraction was repeated twice more, the extracts were concentrated to about 8 liters and the acetone was replaced by hexane. The hexane solution was extracted with warm water and finally the solvent was removed by vacuum distillation. The yield was 1.7% of the original corn oil. The final product contained 52% unsaponifiable matter (completely precipitable by digitonin), 26% triglycerides, 21% free fatty acids and 1% moisture (volatile). Of this final product the daily ration per man was 1.7 g, containing 0.88 g of unsaponifiable matter, dissolved in imitation corn oil. The amount of unsaponifiable fraction given corresponds to that in 55 g/day of original corn oil.

Experimental design. Before beginning the experimental diets, in each experiment the men were maintained for about 6 weeks on a standard "House Diet" (see ref. 10, Table I). At the end of this control period, the matched groups were set up and they began subsistence on the experimental diets following the switchback design, one group starting with one diet and changing later to the second diet, while the other group subsisted on the diets in reverse order. In Exp. N the men were divided into 4 groups. Group W received safflower oil while Group X received corn oil; at the end of 3 weeks, the diets were reversed for another 3 weeks. Groups Y and Z were simi-

TABLE II. Serum Total Cholesterol Values, Means and Stand. Errors, on Corn Oil and 4 Other Vegetable Oils. Observed differences between corn oil and each of the other oils and the corresponding differences predicted from Equation 1, $\triangle$ Chol. = 2.74 $\triangle$S − 1.31 $\triangle$P.

Comparison				Cholesterol values, mg/100 ml				
						△ corn *minus* other oil		Discrepancy, predicted *minus* observed
Corn oil *minus*	Exp.	No. of men	Period, wk	Corn oil	Other oil	Observed	Predicted	
Olive oil	J	14	2	160.7 ± 9.2	187.9 ± 8.4	−27.2 ± 3.6	−17.3	+ 9.9
Cottonseed oil	H	13	4	160.2 ± 9.0	179.5 ±10.1	−19.3 ± 4.2	−11.3	+ 8.0
Sunflower seed oil	J	14	2	160.7 ± 9.2	170.0 ± 9.0	− 9.3 ± 2.8	+ 2.5	+11.8
Safflower oil	N	25	3	162.4 ± 5.5	159.7 ± 5.1	+ 2.7 ± 2.2	+ 9.1	+ 6.4

larly treated except that at the end of the first dietary period 2 other dietary periods were interposed before making the final switchback. In Exp. O after the control period the men were divided into 2 matched groups, A and B, both receiving the same diet for 6 weeks but during the first 3 weeks the men in Group A each received, daily, 1.7 g of unsaponifiable concentrate while Group B had this treatment in the second 3 week period. In all experiments blood was taken on 2 days near the end of each dietary period and serum analyzed for cholesterol (11).

Results. The results from switchback comparisons of olive *vs.* corn oil, cottonseed *vs.* corn oil, sunflower seed *vs.* corn oil, and of safflower *vs.* corn oil (Exp. N) are summarized in Table II. Note that in each experiment the diet was constant except for alternation of 2 types of oil fed at the level of 100 g daily man, representing an average of 28% of total calories.

The most interesting feature of Table II is in the last column, "Discrepancy" between cholesterol differences observed with each pair of oils and the "predicted" difference. Obviously the prediction equation consistently predicts a higher cholesterol value on the corn oil than is actually found. Yet, as shown earlier (10), the prediction equation does not show such a consistent discrepancy with various amounts and kinds of fat (other than corn oil). The other fats and oils seem to form a homogeneous system in which corn oil does not fit precisely. Moreover, the magnitude of the discrepancy with corn oil is substantially the same in all experiments, 6.4 to 11.8 mg/100 ml, and the best estimate is that something in corn oil but absent from the other fats, results in average serum cholesterol about 9 mg% lower than expected when 100 g of corn oil are ingested daily. Alternatively, the other fats tested might contain something absent from corn oil that raises serum cholesterol, this "something" being substantially the same in effect in the other oils.

The results with the unsaponifiable fraction of corn oil are given in Table III. Every man showed a considerable fall in serum cholesterol in changing from house diet to either imi-

TABLE III. Serum Total Cholesterol, mg/100 ml Serum, at Ends of Dietary Periods in Exp. O. Each value is the mean of 4 measurements (duplicates on blood samples drawn on 2 different days).

House diet, control	Imitation corn oil	Imitation corn oil + unsaponifiable
208	165	160
238	184	185
178	156	155
169	147	144
221	164	150
148	126	118
204	179	170
160	135	140
239	202	203
234	196	197
179	147	153
252	200	187
254	196	182
258	194	174
222	170	192
148	132	127
262	198	179
192	165	180
178	131	151
168	140	151
234	171	165
199	154	158
178	140	125
172	138	138
229	174	154
250	206	189
206.7 ± 36.3*	165.8 ± 25.6	162.6 ± 23.2

* ± S.D.

tation corn oil alone or that oil plus the unsaponifiable fraction, the grand average change being –42.5 mg cholesterol/100 ml. Feeding the unsaponifiable fraction was associated with a trivial difference, 3.2 mg%, as compared with the oil mixture alone ($P = 0.17$). It is interesting, however, that the direction of this small difference is the same as in the other comparisons.

Discussion. These experiments demonstrate that the unsaponifiable fraction in corn oil cannot possibly account for the entire decrease in serum cholesterol, when corn oil is substituted for the mixed fats in an American diet. On the other hand, all these experiments consistently point to a small cholesterol-depressing effect of corn oil not accounted for by fatty acid composition, presumably produced by the unsaponifiable fraction, and not in olive, cottonseed, sunflower and safflower oils.

The average serum cholesterol discrepancy resulting from providing 100 g of corn oil in the daily diet is about 9 mg per 100 ml (Table II). From Exp. O the unsaponifiable material corresponding to 55 g of corn oil daily produced a discrepancy of 3.2 mg, suggesting that a discrepancy of 5.8 mg would be expected from the daily ingestion of the unsaponifiable material from 100 g of corn oil. These discrepancies are small, they are not statistically different from each other but they are all in the same direction. We conclude that the unsaponifiable fraction in corn oil probably accounts for a small part of the effect of corn oil on serum cholesterol. Considering both the experiment with isolated unsaponifiable matter and the discrepancies between observed and predicted cholesterol changes when corn oil was interchanged with four other vegetable oils, it is possible to estimate the special serum cholesterol lowering effect of 100 g of corn oil independent of fatty acids and primarily related to unsaponifiable compounds to be between 6 and 12 mg per 100 ml.

The concentrate of unsaponifiable matter obtained from the corn oil contained only about 50% of the total unsaponifiable present in the original oil and there is no way to tell whether the part obtained was more or less active than the part which was lost. We have assumed that the isolated unsaponifiable matter was a representative sample of the total.

These results are not inconsistent with two other series of experiments which have led to opposing conclusions. Beveridge *et al.*(2,8, 13) concluded that most of the effect was due to phytosterols (in the unsaponifiable fraction) in the corn oil, but Ahrens *et al.*(14) removed as much as 80% of the unsaponifiable material from corn oil and failed to demonstrate an alteration in the effect of the oil on the serum cholesterol. Data have not yet been published to support the conclusions of Beveridge *et al.* and the evidence from Ahrens *et al.* actually does not rule out the possibility of an effect of the magnitude found here.

Farquhar and Sokolow(15) have shown that the effects of plant sterols and of poly-ene fatty acids in lowering serum cholesterol are additive and this is also indicated here. The present experiments indicate that if practically all of the unsaponifiable material in corn oil consists of phytosterols, something like 3 g of such phytosterols ingested per day would produce a fall of the serum cholesterol of the order of 10 mg per 100 ml. This is, in fact, about the least dosage of sitosterol which is reported to result in a discernible change in the serum cholesterol(16).

In a recent trial corn oil tended to produce more cholesterol lowering than an equal amount of safflower oil(4). This finding, too, is consistent with the present results.

Summary. 1) Comparison of corn oil with 4 other oils of the linoleic-oleic acid type consistently indicated that corn oil in the diet produced slightly lower serum cholesterol values than those predicted, on the basis of fatty acid composition, by the multiple regression equation previously described. The mean discrepancy in 4 sets of switchback dietary comparisons with 13 to 25 men each and using 100 g of each oil in the daily diet was 9 mg/100 ml. 2) The source of this discrepancy was sought by feeding in a switchback experiment a daily supplement containing 0.88 g of unsaponifiable matter from corn oil, equivalent to that in 55 g of corn oil, added to a diet containing 140 g of fat of which 100 g was a mixture of safflower and cottonseed oils imitating corn oil. 3) Addition of unsaponifiable

matter produced a mean decrease of 3.2 mg of cholesterol/100 ml of serum. Although this effect is statistically non-significant, it matches the discrepancy observed in the earlier comparisons. It is concluded that the unsaponifiable matter of 100 g of corn oil lowers serum cholesterol by something between 6 and 12 mg 100 ml.

1. Ahrens, E. H., Tsaltas, T. T., Hirsch, J., Insull, W., *J. Clin. Invest.*, 1955, v34, 918.

2. Beveridge, J. M. R., Connell, W. F., Mayer, G. A., *Canad. J. Biochem. Physiol.*, 1956, v34, 441.

3. Keys, A., Anderson, J. T., Grande, F., *Lancet*, 1957, v1, 66.

4. Armstrong, W. D., van Pilsum, J., Keys, A., Grande, F., Anderson, J. T., Tobian, L., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 302.

5. Malmros, H., Wigand, G., *Lancet*, 1957, v273, 1.

6. Shapiro, W., Estes, E. H., Hilderman, H. L., *Am. J. Med.*, 1957, v23, 898.

7. Jones, R. J., Reiss, O. K., Huffman, S., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 88.

8. Beveridge, J. M. R., Connell, W. F., Mayer, G. A., *Fed. Proc.*, 1957, v16, 11.

9. Keys, A., Anderson, J. T., Grande, F., *Lancet*, 1957, v1, 787.

10. ———, *ibid.*, 1957, v2, 959.

11. Anderson, J. T., Keys, A., Grande, F., *J. Nutrition*, 1957, v62, 421.

12. Assn. of Life Insurance Medical Directors. Medico-actuarial Mortality Investigations; N. Y., Assn. Life Insur. Med. Dir. Actuarial Soc. America, v1, 1912.

13. Beveridge, J. M. R., Connell, W. F., Mayer, G. A., Haust, H., *Circulation*, 1957, v16, 491.

14. Ahrens, E. H., Hirsch, J., Insull, W., Tsaltas, T. T., Blomstrand, R., Peterson, M. L., *Lancet*, 1957, v1, 942.

15. Farquhar, J. W., Sokolow, M., *Circulation*, 1957, v16, 494.

16. Shipley, R. E., *Trans. N. Y. Acad. Sc.*, 1955, v18, 111.

Received March 26, 1958. P.S.E.B.M., 1958, v98.

Failure to Demonstrate Toxic Factors in the Serum of Irradiated Rats.* (24070)

ROBERT D. CAMPO,† VICTOR P. BOND, AND EUGENE P. CRONKITE

St. John's University and Medical Department, Brookhaven National Laboratory, N. Y.

Although circulating toxic humoral factors may play a role in radiation-induced illness, little unequivocal evidence supports such a factor(1). It has been demonstrated that serum from irradiated rabbits, incorporated into the media for growing chick heart fibroblasts and *Escherichia coli* depressed their rate of cell division(2). Van Dyke and Huff(3) reported that, in rats parabiotically united before irradiation of one of the parabionts with 900 r of X-radiation, epilation of both parabionts occurred. Non-irradiated parabiotic rats and single rats given 900 r did not show this effect. Lavik *et al.*(4), assumed that toxic factors were dialyzable but was not able to prolong, by *in vivo* dialysis, the lives of dogs which were given 500 r of whole body X-radiation. Whole blood taken at various time intervals post-irradiation from rats given supralethal amounts of whole body Co^{60} gamma radiation failed to cause weight changes or mortality in rats injected intravenously with this material(5). Recently Edelmann presented evidence indicating existence of lethal toxic substances in blood of adrenalectomized rats.‡ He reported that adrenalectomized rats and mice, injected intraperitoneally and intravenously with serum, plasma, and whole blood from other adrenalectomized irradiated rats (1000 r) died sooner than adrenalectomized animals which received blood from non-irradiated adrenalectomized donors.

The present experiments were initially de-

* This work was supported by U. S. Atomic Energy Com.

† Some data in this report were included in thesis submitted to Graduate School of St. John's University, N. Y., in partial fulfillment for degree of Master of Science. Present address: Predoctoral Fellow, Rockefeller Institute, N. Y.

‡ Reported in *Fed. Proc.*, 1955, 42.