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Evidence of the Specificity of the Intracutaneous Pollen Test in Man.

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Duplication of positive skin reactions to pollen extract was possible in most of the patients when 2 tests were made simultaneously, or when 2 or more weeks elapsed between the 2 tests. The pollens employed included a gramineae, a chenopodiaceae and 2 compositae. The majority of the individuals were positive to but a few pollens though some were positive to nearly all pollens.

Six species of *Artemisia* and 7 of *Atriplex* were tested on a series of patients. We have demonstrated that an individual may react to but 1 or 2 species of the 6 or 7 tested. Eighteen individuals reacted to but one of the 2 subspecies tested, while 6 patients reacted to the other subspecies.

In combination with the factor of accuracy previously discussed, these findings are considered evidence of the specificity of the intracutaneous test and, *a priori,* of the reacting substances in the pollen extract.

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A New Dietary Deficiency With Highly Purified Diets. III. The Beneficial Effect of Fat in the Diet.*

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Some years ago Osborne and Mendel[1] reviewed the work which had been done on low fat diets, and from an experiment with 6 young male rats concluded that "if true fats are essential for nutrition during growth, the minimum necessary must be exceedingly small." In view of the fact that their experimental animals were compared with the animals reared on a diet high in fat but low in protein, it seemed probable that both the controls and the experi-

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mental groups were subnormal, thus leaving the question unsettled.†

It has been observed in this laboratory that our lard free diet 316 (Table I) is uniformly inferior to the high fat diet 232. Recently

TABLE I.
Composition of Diets.

Ingredients	Diet 232	Diet 316	Diet 519	Diet 520	Diet 522	Diet 525	Diet 526	Diet 527	Diet 528	Diet 529	Diet 533	Diet 534	Diet 535
Casein (commercial)	32	24.1		25.8		30.0	30.0	30.0	30.0	30.0	27.0	25.0	29.5
Casein (pure)			24.0		30.0								
Salts 185	4	3.6	3.8	3.8	4.5	4.5	4.5	4.5	4.5	4.5	4.0	4.0	4.0
Cornstarch (cooked)	40	72.3											
Sucrose (commercial)				70.4		45.5	45.5	45.5	45.5	45.5	59.0	66.0	48.5
Sucrose (purified)			72.2		45.5	20.0							
Lard	22				20.0		20.0				10.0	5.0	
Butter fat								20.0					
Corn oil (Mazola)									20.0				
Coconut oil (refined)										20.0			
Stearic acid													
Fatty acid from lard													18.0
Cod liver oil (Patch)	2												

† We have recently shown that their diet designated by us as our Basal Ration (Casein 18, cornstarch 54, lard 15, butter fat 9, salts-185 4) does not permit normal growth and ovulation in female rats.

TABLE II.
Showing the superiority of the high fat diet 232 over the low fat diet 316.

Diet	No. of animals	Average age at rupture of vaginal membrane	Average age at first oestrus	Total No. of ovulation cycles up to 90 days of age	Average No. of cycles per animal	Average wt. at 90 days
		days	days			gm.
232 + 0.7 g. yeast daily	15	39	41	136	9.1	206
316 + 0.7 g. yeast + 2 drops cod liver oil daily	15	47	54	63	4.2	188

TABLE III.
The Effect of Fat in the Diet.

Diet	Animal No.	Age at rupture of vaginal membrane	Age at 1st oestrus	Ovulation cycles. Length in days.	No. of cycles to 90 days	Wt. at 90 days	Average wt. of group at 90 days
		days	days			gm.	gm.
519 +							
1.0 gm. ether extracted yeast	W4936	44	48	5, 8, 7, 6, 13, 8, 5.		132	
	W4942	45	45	5, 5, 5, 4. 6, 5, 5, 6, 5, 8	23	136	136
3 drops cod liver oil	W4939	42	42	10, 6, 2, 3, 8, 7		142	
522 +							
1.0 gm. ether extracted yeast	W4958	41	42	5, 5, 6, 5, 6, 5, 5, 6, 6, 6		189	
	BH4957	41	48	6, 7, 5, 5, 5, 7, 5, 7	28	182	182
3 drops cod liver oil	BH4956	43	43	4, 8, 5, 7, 6, 5, 5, 5, 5, 6		174	

a carefully controlled experiment has been conducted to compare diet 316 plus 2 drops of cod liver oil (Patch) daily with diet 232. The groups of 15 females were made up of littermate sisters. Each diet was supplemented by 0.7 gm. yeast (Fleischman) daily. Table II gives the average results. It will be seen that the performance on the 2 diets is very different, the animals on diet 316 being retarded in growth, sexual maturity and ovulation. In fact, the rate of ovulation is decreased to less than 50% of its normal value. (The growth, maturity and ovulation here recorded for animals on diet 232 is equal to the normal on natural foods.)

TABLE IV.
Comparing the value of various fats as a supplement to low fat diets.

Diet	Animal No.	Age at rupture of vaginal membrane	Age at 1st oestrus	Ovulation cycles	No. of cycles to 90 days	Wt. at 75 days	Wt. at 90 days	Average wt. of the group at 90 days
		days	days	days		gm.	gm.	gm.
520+	B6402	78	80	9		144	162	
0.7 g. yeast	W6409	71	72	7, 4, 6, 5		114	132	
3 drops cod	G6422	78	80	6		118	138	
liver oil	W6429	68	72	7, 5	14	120	138	144
	BH6436	76	—	— — — —		112	138	
	W6393	63	63	5, 6, 6, 4, 5, 5		140	156	
525+	BH6403	54	54	7, 4, 7, 5, 5, 5, 5		170	191	
0.7 g. yeast	B6424	44	44	5, 6, 7, 10, 9, 5		166	178	
3 drops cod	W6404	61	62	4, —	41	142	158	177
liver oil	W6431	53	56	4, 7, 5, 5, 5, 5, 5, 5		170	178	
	W6411	44	44	3, 6, 6, 6, 5, 5, 5, 6, 6		155	168	
	W6394	40	40	5, 5, 7, 6, 5, 5, 5, 5, 5, 5		166	190	
526+	W6401	56	56	6, 5, 9, 7, 6, 8		126	140	
0.7 g. yeast	W6405	55	68	6, 6, 6, 4, 5		170	190	
3 drops cod	W6412	36	36	8, 5, 4, 4, 5, 4, 4, 4, 4, 4, 8, 4	31	150	160	168
liver oil	B6425	51	81	— — — —		155	168	
	W6363	57	69	8, 6, 7, 6		150	174	
	B6468	53	53	13, 16, 10		155	174	
527+	W6406	56	85	9		120	150	
0.7 g. yeast	B6413	48	49	14, 6, 7, 5, 6, 6		168	188	
3 drops cod	B6426	45	48	6, 16, 11, 6, 5		128	150	
liver oil	W6433	54	54	11, 8, 7, 5, 5, 6	25	140	160	170
	B6469	54	61	5, 11, 7		135	152	
	BH6395	48	54	13, 10, 11, 11		185	220	
528+	W6407	76	81	9, 7		113	140	
0.7 g. yeast	W6420	59	78	6, 13		126	140	
3 drops cod	W6427	52	58	8, 10, 4, 5, 5, 6	22	155	178	164
liver oil	B6434	43	43	4, 6, 5, 6, 5, 5, 5, 5, 5, 5		165	188	
	B6470	72	89	— — — —		136	170	
	BH6396	55	77	8, 8		160	170	
529+	W6408	76	—	— — — —		80	104	
0.7 g. yeast	W6421	95	—	— — — —		80	98	
3 drops cod	W6428	76	—	— — — —	1	86	100	106
liver oil	BH6435	76	77	— — — —		104	120	
	W6471	92	—	— — — —		78	82	
	BH6397	77	77	12		108	120	
520+	W6410	58	61	5, 4, 6, 5, 5, 5, 5		150	160	
0.7 g. yeast	B6423	51	55	8, 12, 5, 7, 4, 6		175	196	
3 drops cod	W6430	69	77	6, 10		150	160	
liver oil	BH6437	57	59	6, 5, 6, 6, 5, 5, 5	26	164	160	171
10 g. fresh let-	W6472	77	87	— — — —		135	140	
tuce daily	B6398	61	68	11, 7, 4, 6		186	210	

TABLE V.
A further study of the value of lard and butter as supplements to a low fat diet.

Diet	Animal No.	Age at opening	Age at 1st oestrus	Ovulation cycles	Total No. of cycles to 90 days	Wt. at 60 days	Wt. at 90 days	Average wt. of group at 90 days
		days	days	days		gm.	gm.	gm.
520+	W7185	46	47	14, 12, 16		120	140	
0.7 g. yeast	W7200	48	50	9, 27		126	122	
3 drops cod	W7218	55	55		9	149	150	133
liver oil	BH7190	53	53			130	130	
daily	B7176	41	44	11, 8, 48		130	132	
	B7193	59	62	32		120	124	
525+	W7184	45	45	4, 5, 10, 13, 4, 4, 4		149	168	
0.7 g. yeast	W7199	48	57	4, 5, 10, 4, 3, 6	44	160	180	179
3 drops cod	W7217	48	48	11, 5, 5, 5, 5, 4, 8, 7		164	198	
liver oil	GH7189	46	46	11, 5, 5, 4, 4, 4, 4, 4, 4		140	172	
daily	GH7180	40	46	4, 5, 5, 6, 4, 6, 4, 4, 6		160	202	
	B7192	50	50	6, 8, 7, 5		149	170	
526+	BH7188	45	45	5, 9, 14, 5, 10		142	170	
0.7 g. yeast	W7219	45	45	15, 9, 7, 5, 6, 6		180	196	
3 drops cod	G7225	46	46	12, 5, 5, 5, 4, 5, 4, 4		149	160	
liver oil	W7178	44	45	9, 6, 9, 5, 6, 4, 7	38	172	194	180
daily	BH7183	46	47	7, 12, 5, 4, 5, 6, 5		140	180	
	BH7196	50	50	10, 11, 12, 44		158	180	
533+	W7187	45	45	4, 9, 5, 7, 5, 5, 4, 7		152	172	
0.7 g. yeast	BH7202	43	43	8, 5, 7, 5, 6, 6, 5, 5		138	152	
3 drops cod	B7224	42	42	4, 7, 6, 4, 6, 5, 6, 5, 8		140	140	
liver oil	BH7177	39	39	5, 6, 5, 9, 5, 5, 13	46	160	176	161
daily	G7182	35	35	7, 5, 6, 6, 5, 5, 5, 5, 5, 5		142	158	
	BH7195	51	70	7, 5, 5, 5		158	170	
534+	W7186	47	50	8, 5, 6, 6		150	176	
0.7 g. yeast	W7201	39	43	8, 6, 13, 8, 8, 5, 5, 5		160	164	
3 drops cod	B7223	47	47	5, 5, 5, 4, 5, 4, 5, 4		140	164	
liver oil	BH7191	49	52	7, 9, 5, 5, 5, 5, 5	39	138	142	163
daily	GH7181	40	44	5, 5, 5, 5, 10, 5, 5, 6		148	165	
	B7194	52	56	5, 6, 5, 4, 5		142	168	

By the substitution of sucrose for starch and of pure casein for commercial casein, the fat content of the diet has been further reduced (starch has usually 0.5 to 0.6% non-extractable fat and commercial casein carries a variable amount of butter fat). While working with these purer and simpler diets (Diets 519, etc., Table I) it was found that by the addition of lard to the diet, ovulation was always somewhat improved and growth was markedly improved. (Table III.) In fact, the animals which received the high fat diet 522 may be considered about normal in weight and ovulation, while those on the fat free diet 519, are greatly stunted.

In pursuing this study further a comparison has been made of the effects of various fats on the performance of young female rats. All these experiments have been done with wire bottom cages except for the comparison of diets 316 and 232.

The results summarized in Table IV show that 4 fats, lard, butter, cocoanut oil, and corn oil, when added to the sugar-casein diet, all significantly improved growth and ovulation during the first 90 days of life. The improvement was practically identical with that secured by the addition of lettuce. It may be noted that lard is not inferior (in fact, is slightly superior) to the other fats employed. This led us to repeat the experiments with lard (Table V), employing 5%, 10%, and 20% levels of this fat in contrast with littermate sister controls on the fat free diet 520 and on butter (diet 526). The ovulation history was virtually identical in all these groups but the growth in the groups receiving the lower levels of lard (5% and 10%) was slightly inferior.

Shortly after their 90th day of life an attempt was made to breed all females in these groups and to study the lactation performance on these diets. In all cases, during lactation the yeast dosage was

TABLE VI.
Lactation performance of animals on sugar-casein diet supplemented with various fats.

Diet	No. of mothers	No. of young to be suckled	No. of young weaned	% weaned	Aver. birth weight in gm.	Aver. weaning weight in gm.	% increase weight of young
520+ 0.7 gm. yeast 3 drops cod liver oil	1	6	6	100.0	5.2	20.6	295
520+ 10 gm. lettuce 0.7 gm. yeast 3 drops cod liver oil	6	36	30	83.0	5.1	26.0	410
525+ 0.7 gm. yeast 3 drops cod liver oil	3	14	13	93.0	5.7	28.6	402
526+ 0.7 gm. yeast 3 drops cod liver oil	6	36	31	86.0	5.2	36.7	606
527+ 0.7 gm. yeast 3 drops cod liver oil	3	18	15	83.0	4.8	30.0	524
528+ 0.7 gm. yeast 3 drops cod liver oil	4	24	23	96.0	5.3	32.6	516

increased 3 times in order to give the sucklings adequate vitamine B. Table VI gives the essential results. One may note that on the lard diets the worst rather than the best performance was exhibited. The butter diet is in respect to lactation easily the best of the series, furnishing, in fact, normal weaning weight. We are not yet prepared to explain the favorable influence of butter.

It will be noted that the addition of stearic acid in all cases stunted the animals badly and prevented the attainment of sexual maturity.

It seemed desirable to see if the valuable substance in fats could be located exclusively in any one of the 3 well recognized fractions —the fatty acids, the glycerol, or the non-saponifiable matter. Lard was saponified in 20% alcoholic potassium hydroxide on the steam bath and the non-saponifiable matter was extracted, washed and recovered in the usual way. The fatty acids were precipitated by dilute hydrochloric acid and recovered. Pure glycerol was fed instead of attempting a recovery of that substance from the aqueous layer left from the fatty acid. The comparison of these 3 mater-

TABLE VII.

Comparing the value of three fractions of lard in supplementing low fat diets.

Diet	Animal No.	Age at rupture of vaginal membrane	Age at first oestrus	Ovulation cycles to 90 days	Number of cycles to 90 days	Wt. at 60 days	Wt. at 90 days	Average wt. of group at 90 days
		days	days			gm.	gm.	gm.
520+	W7301	57				124	136	
0.7 g. yeast	W7304	63				130	138	
3 drops cod	BH7283	36	48	8, 4		156	150	
liver oil	W7290	65			9	120	112	133
N. S. M. from	B7279	45	50			119	139	
1.2 g. lard	W7326	36	54			114	124	
daily								
535+	BH7278	45	51	5, 5, 4, 4, 5, 4, 4, 4, 4		160	192	
(fatty acid	W7289	56	56	4, 8, 4, 5, 6, 4, 5		141	157	
from lard)	BH7303	50	50	17, 5, 5		132	152	
3 drops cod	BH7282	45	46	8, 10, 5, 20	31	154	176	158
liver oil	W7299	44	44	8, 6, 5, 5, 7, 6, 5, 5		144	162	
daily	W7327	59	94			100	108	
520+	BH7277	40	40	6, 7, 6, 16		120	122	
0.7 g. yeast	W7288	54				126	130	
3 drops cod	BH7300	51	51	5, 4, 4, 5		132	142	
liver oil	BH7281	57	74	6, 8	17	140	132	128
5 drops gly-	W7298	43	43	27, 39		128	132	
cerol daily	W7328	46	46	7, 5, 11, 12, 7		108	108	

ials was made and the results summarized in Table VII. They show that the group receiving the fatty acids was significantly superior to those receiving the non-saponifiable matter and the glycerol, neither of which could be described as improved by the addition, and they clearly indicate that the favoring substance has not been seriously hurt by the process of saponification.

Conclusions. (1) The highly purified and almost fat free diets 519 and 520 which give subnormal growth and ovulation, except when supplemented with small amounts of beef liver, lettuce or lard, can also be adequately supplemented by other fats, in particular by cocoanut oil, corn oil or butter. (2) The favorable substance in fats—possibly a new vitamine (F)—unlike Vitamines A, D and E, is not concentrated in the non-saponifiable fraction. It can be recognized in the fatty acid portion after saponification.

[1] Osborne and Mendel, *J. Biol. Chem.*, 1920, xlv, 145.