Summary. Protein extracts of the neural plate plus underlying chordamesoderm induce the presumptive epidermis of the gastrula to form neural tubes. However no differences were obtained in the structure of the neural tubes which were induced by extracts of anterior as compared with posterior regions of the neural plate and underlying chordamesoderm. Preparations of the anterior and posterior regions of the neural plate and underlying chordamesoderm which were killed by freezing and subsequent drying induced neural tubes in the explanted presumptive epidermis of the gastrula. Quantitative differences in the frequency of induction were obtained with the posterior neural plate and underlying chordamesoderm inducing with the greatest frequency.

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Further Report on Malignancy Observed in Rats Injected with Crude Ether-Extracted Wheat Germ Oil.

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In previous papers from this laboratory[1-3] the presence of a carcinogenic substance has been demonstrated in crude ether-extracted wheat germ oil. An interesting observation is that some of these tumors developed from feeding this oil, either by a forced feeding method or by mixing the oil with the diet. However, some were also produced by intraperitoneal injection of the oil. Since that time, several investigators[4-9] have attempted to produce neoplasms by the feeding of similar oils, but all have been unsuccessful. However, nothing has been further reported upon the production of malignant tumors by injection of a crude wheat germ oil.

In the past 3 years our attempts to produce tumors by feeding crude ether-extracted wheat germ oil have, as with other investigators, resulted in failure. On the other hand, by injection we have been able to produce a substantial number of tumors with some of the oils available, a fact which has been confirmed by the similar results of another investigator.[10]

Materials and Methods. Four strains of rats, Wistar, Buffalo, a hooded variety of our own breeding, and a strain of Wistar rat supplied by Eli Lilly and Company were used. Four different oils were used also. One was a percolated oil with the sediment removed, supplied by Eli Lilly and Company. This oil is identified by the letter L in the table. The 3 other oils were prepared by us by a Soxhlet type of extraction, one for 8 hours (8E), one for 12 hours (12E), and one for 24 hours (24 Ma). These were used as prepared, the sediment shaken each time before use, except that in one sample of the 24-hour oil the sediment was removed before use (24 MaC). Two series of control oils were also used. One was a purified expressed wheat germ oil supplied by Eli Lilly and Company. The other was sesame oil, divided into 3 parts, one part of which was used plain, another contained 2% of starch in suspension, and a third con-

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TABLE I.
Results of Injections of Crude Wheat Germ Oil.

No. of animals	Strain	Oil No.	No. of tumors	Type of tumor	Days elapsed before autopsy	% bearing tumors
1	Hooded	24 Ma	0			
2	Buffalo	24 Ma	0			
2	"	24 MaC	0			
2	Hooded	8E	0			
3	Wistar	8E	1	Ca	223	33
8	"	24 Ma	2	1 Ca	545	25
				†	674	
3	"	24 MaC	0			
3	"	12E	0			
3	"	L	2	2 Sa	249, 353	66
10	" *	L	5	5 Sa	220, 289, 299 478, 588	50
2	" *	24 Ma	0			
2	" *	12E	1	Ca	671	50
3	"	Sesame	0			
3	"	Sesame, 2% starch	0			
3	"	Sesame, 2% starch, 2% sterols	1	Sa	555	33
5	"	Pressed wheat germ oil	0			
				Total rats		27
				Wistar rats only		32

* Of Purdue stock supplied by Eli Lilly & Company.
† Atypical cell tumor not definitely classified.

tained 2% of starch and 2% of wheat germ sterols. The purpose of these was to see if some of the known constituents of the wheat germ oil used had any effect.

Each animal received 1 cc of an oil intraperitoneally once a week until a palpable mass was detected in the majority of the animals. This required 4 injections. The oil was then given but once in 2 weeks. After about 30 injections the oil was usually exhausted, and the animals were allowed to live some time without further treatment. The usual laboratory diet was given throughout the experiment.

Results. After the first 4 injections of wheat germ oil there was palpable in the abdomen of all animals a large, rather firm mass. To determine what this mass was, 3 animals not included in this report were injected with crude wheat germ oil for 4 weeks and sacrificed on the fifth week. Autopsy revealed that the mass was caused by a peritonitis. The intestines, spleen, liver, and bladder were all enveloped and connected with fibrous adhesions giving a solid mass which could not easily be separated by blunt dissection. No considerable amount of oil was to be found anywhere within the mass.

The injections were carefully continued on the other animals. After 7 or 8 injections the mass as a rule became quite hard. After about 15 injections one-third of the animals developed a sinus penetrating through the peritoneal wall, and skin, which in some cases evidenced a purulent discharge and in some cases remained clean. No oil, however, could be found oozing from such wounds. These healed as a rule, leaving a hernia in a few cases. The injections were continued every other week unless the life of the animal seemed endangered when alternate injections were omitted. Malignancy was shown in animals that came to autopsy in from 220 to 670 days.

The control oils gave negative findings showing neither the peritonitis nor the lesions which were obtained with the crude wheat germ oil.

Table I gives in detail the final results of this series of experiments.

Malignant tumors were obtained in the two Wistar strains of rats only. Combining these, a total of 32% of the Wistar animals on all crude wheat germ oils developed malignant tumors. It is worthy of note perhaps, that the one sesame oil control rat which also succumbed to a malignant tumor, was one in which 2% of wheat germ oil sterols were

dissolved and 2% starch was suspended.

The control animals lived as long as the experimental animals (353 to 647 days). There were no spontaneous tumors throughout the colony of over 500 rats from which most of the experimental animals were taken.

One experimental animal was sacrificed after 362 days and the tumor found carried through 6 generations of transplants.

Discussion. It is believed that these results definitely indicate the presence of a carcinogenic substance in crude ether-extracted wheat germ oil. Because of the presence of adhesions within the abdomen it might be argued that the malignancy was caused solely by irritation. But, if one considers that while all of the crude wheat germ oils used produced similar adhesions, yet the percentage of actual malignancy varied widely with different oils, this conclusion appears unwarranted. Indeed, this variation of tumor growth with different batches of crude wheat germ oil made by extraction with ether, lends support to our belief that a carcinogenic substance is present in some oils and absent in others. We are, however, unable to state as yet all the conditions essential to reproduce consistently these results with a wheat germ of any source.

Summary. Malignant tumors have been produced in, on an average of, 32% of Wistar rats injected with crude wheat germ oil made by extraction with ether. With one batch of oil, tumors resulted in 66% of the injected rats. The tumors were preponderantly sarcomas but carcinomata were induced in 3 rats.

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Effect of Pseudopyridoxine on Rat and Yeast Growth.*

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In a recent report Snell, Guirard, and Williams[1] demonstrated the presence of a substance in natural products which surpasses pyridoxine in its physiological activity on *Streptococcus lactis* cultured on a pyridoxine-free medium. The substance had properties similar to pyridoxine, hence was called pseudopyridoxine. Tissues and urine were found to contain pseudopyridoxine, the amount depending on the quantity of pyridoxine ingested. These workers suggested that the compound is a metabolite of pyridoxine. Snell[2] showed that pseudopyridoxine was formed when pyridoxine was autoclaved with the basal culture medium for *S. lactis*. The amount formed increased as the period of heating was increased, and was due to the interaction of pyridoxine with amino acids during autoclaving.

Before the paper of Snell *et al.*[1] appeared it had been observed in this laboratory that the recovery of pyridoxine after various chemical treatments was very high as measured by the *Lactobacillus casei* method of Landy and Dicken.[3] Since this test organism responds to pseudopyridoxine in a manner

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