The amount of urine in the samples was carefully measured. The found average for the whole group was 1.066 cc. with a S.D. 1.42. The determinations of total nitrogen were

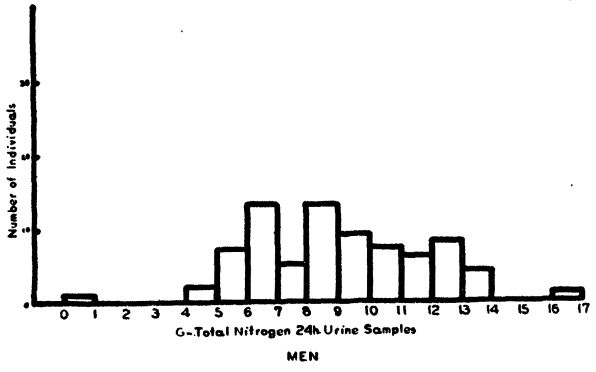



FIG. 3

made with the macro-Kjeldahl technique (*9*), using Gunning's modification (*8*). The determinations, carried out in duplicate in 80 per cent of the cases, checked well.

## RESULTS

The histograms for the whole group, the female group, and the male group are shown in Figs. 1, 2, and 3, respectively.

In Fig. 1 the values are expressed in grams of nitrogen and in calculated metabolized proteins (N × 6.25). Table 1

TABLE 1

| | No. Individuals | Averages (grams N/day) | S.D. | N × 6.25 | Modes (grams N/day) |
|---|---|---|---|---|---|
| Whole group..... | 194 | 8.03 | 2.80 | 50.2 | 6–7 |
| Female..... | 118 | 7.46 | 2.56 | 46.6 | 6–7 |
| Male..... | 76 | 8.89 | 2.74 | 55.6 | 6–9 |

shows the averages, standard deviations, calculated metabolized protein corresponding to the averages, and the modes of the distributions of the found values.

## References


1. BIDDER, F., and SCHMIDT, C. *Die Verdauungssaefte und der Stoffwechsel* Mitau: Reyher, 1852.
2. BIGWOOD, E. J. *Guiding principles for studies on the nutrition of populations.* Geneva: League of Nations, 1939.
3. BOUSSINGAULT. *Ann. Chim. Phys.*, 1839, **2**, 113.
4. CARTAYA, J. A. M. D. Thesis, Universidad Central Venezuela, 1943.
5. DE VENANZI, F. *Rev. Sos. Est. Med.*, 1940, May, 5–45.
6. DE VENANZI, F. *Rev. Min. Sanidad Asist. Soc.*, 1945, **10**, 251.
7. GARCIA AROCHA, H. *An. Inst. Med. Exp.*, 1942, **1**, 81–139; DE VENANZI, F. *Rev. Min. Sanidad Asist. Soc.*, 1942, **7**, 847.
8. GUNNING. *Z. anal. Chem.*, 1889, **28**, 188.
9. KJELDAHL. *Medd. Carlsberg Lab.*, March, 1883; *Z. anal. Chem.*, 1883, **22**, 336.
10. LIEBIG, J. *Organic chemistry.* London: Taylor and Walton, 1840; *Die Thierchemie.* Braunschweig: Vieweg, 1842.
11. VOIT, C. *Physiologisch-chemische Untersuchungen.* Augsburg: Rieger, 1857; BISCHOFF, and VOIT, C. *Die Gesetze der Ernahrung des Fleischfressers.* Leipzig: Winter, 1860; VOIT, C. *Physiologie des allgemeinen Stoffwechsels und der Ernahrung.* In HERMANN, L. *Handbuch der Physiologie,* 1881.
12. ————. The most suitable methods of detecting malnutrition due to the economic depression. *Quart. Bull. Hlth. Org. League Nat.*, 1932.


# Heterologous Transplantation of Human Tumors


MACHTELD E. SANO

*Agnes Barr Chase Surgical Research Foundation, Temple University School of Medicine and Hospital, Philadelphia*



Two human tumors of the same type, both questionable lymphosarcomata or leukemias, were transplanted into mice after passage through tissue culture. One animal developed a tumor which metastasized to the liver and was identical to the human tumor. Another developed a similar tumor at the site of inoculation, but the cells infiltrated the adjacent breast tissue, initiating in it an adenocarcinoma which metastasized rapidly. Another mouse presents a leukemic picture with an absolute white count of 37,000. The suspected lability of these difficult-to-diagnose lymph-node tumors is thus experimentally demonstrated. Preliminary passage through tissue culture permitted transplantation of the human tumor to the animal without previous passage through the anterior chamber of the eye.


# Inhibition of Heating and Lipolysis in Seeds


MARJORIE Z. CONDON, F. R. ANDREWS
MADELINE G. LAMBOU, and A. M. ALTSCHUL[1]

*Southern Regional Research Laboratory,[2] New Orleans, Louisiana*


Heating and deterioration of moist seeds has constituted a problem in those processing industries where seeds must be stored in bulk for extended periods of time. Damage resulting from heating is manifested not only by visible discoloration of the seeds but also by reduction of the quantity and quality of the processed products. For example, cottonseed which has been damaged by heating during storage will yield less oil than prime cottonseed, and the oil which is obtained will contain an increased percentage of free fatty acids.

Heating in seeds during commercial storage is minimized by one of the following procedures: (1) predrying prior to storage, (2) forced aeration during storage, or (3) stacking bagged seeds in a manner that provides natural circulation of air in the interspaces. Reduction or prevention of deterioration may also be effected by treating the seeds with chemicals to inhibit the biological processes which are responsible for heating and deterioration (*1, 3, 4, 5*). An investigation has been made to determine the effectiveness of a wide variety of chemicals as such inhibitors. Preliminary results of this survey are presented here, and detailed reports on the calorimetric method used and its application to problems of seed storage will appear elsewhere.


[1] The authors wish to acknowledge the invaluable aid given them by the many chemical manufacturers who suggested and furnished many of the compounds tested to date. The free fatty acid determinations were made by Miss Claire Lesslie.


[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

The test is performed by placing seeds, which have been artificially moistened and treated with the chemical whose inhibitory power it is desired to test, into calibrated Dewar flasks and drawing air through them at a rate which will support maximum heating. The temperature within the seeds is determined periodically by means of a thermocouple and manually-operated or recording potentiometer. After one week of storage under these conditions, the seeds are removed from the flasks and examined for other evidence of deterioration, e.g. content of free fatty acids in the case of oil seeds.

To compensate for differences in biological activity resulting from variations in age, history, variety, and type of sample, the response of the seeds treated with any chemical or group of chemicals is compared simultaneously with an untreated sample of the same seed having the same moisture content, and with a sample of the same seed having the same moisture content but which had been treated with a standard quantity of ethylene chlorhydrin.

Response curves obtained in a typical experiment are shown in Fig. 1. In this graph the effect of treating flaxseed conditioned to 22 per cent of moisture (wet basis) with different concentrations of diethyl oxalate is compared with the standard ethylene chlorhydrin treatment. A concentration

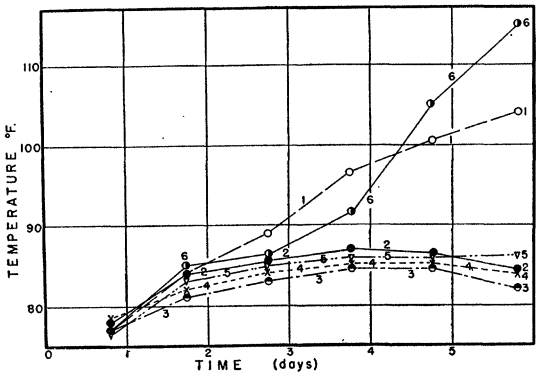



FIG. 1. Effect of ethylene chlorhydrin and diethyl oxalate on heating of moist flaxseed.

| No. | Inhibitor | Concentration % |
|---|---|---|
| 1 | | |
| 2 | Ethylene chlorhydrin | 0.38 |
| 3 | Diethyl oxalate | 0.35 |
| 4 | " " | 0.28 |
| 5 | " " | 0.24 |
| 6 | " " | 0.16 |

of 0.24 per cent[3] diethyl oxalate was found to inhibit heating as effectively as 0.38 per cent ethylene chlorhydrin. It should be noted that treatment of the seed with 0.16 per cent diethyl oxalate stimulated the evolution of heat so that temperatures above that of the untreated control were observed. As will be shown (Table 1), the latter concentration of diethyl oxalate also stimulated lipolysis. The phenomenon of stimulation or inhibition as a function of concentration has been repeatedly observed in seed storage investigations (*2–4*) and appears to be an inherent property of many substances normally considered as inhibitors (*7*). After the seeds have been in the Dewar flasks for 6 days, they are removed and analyzed for free fatty acids content. The results of determination of free fatty acids corresponding to the calorimeter data in Fig. 1 are recorded in Table 1, from which it is apparent that diethyl oxalate is more effective as an inhibitor of heating than of lipolysis.

[3] Concentration is expressed in terms of weight of inhibitor per dry weight of treated seeds.

TABLE 1

EFFECT OF TREATMENT OF MOIST FLAXSEED WITH DIETHYL OXALATE AND ETHYLENE CHLORHYDRIN ON DEVELOPMENT OF FREE FATTY ACIDS IN THE SEED OIL

| Inhibitor | Concentration* (%) | Free fatty acid content† (%) |
|---|---|---|
| None | | 3.65 |
| Ethylene chlorhydrin | 0.38 | 0.79 |
| Diethyl oxalate | 0.35 | 1.05 |
| " " | 0.28 | 1.63 |
| " " | 0.24 | 2.20 |
| " " | 0.16 | 4.88 |

* Weight of inhibitor per weight of dry seed.

† Free fatty acids content of original seeds, 0.78 per cent; free fatty acids determined after storages for 6 days.

By the use of the above-illustrated method it has been possible to determine the concentration of inhibitor which will give approximately the same degree of inhibition as the standard concentration of ethylene chlorhydrin. All of the chemicals have, therefore, been tested in concentrations of approximately 0.38 per cent. Those which had less activity than ethylene chlorhydrin when applied in this concentration were rejected, while those exhibiting activity equal to or better than the

TABLE 2

RELATIVE EFFECTIVENESS OF VARIOUS CHEMICALS AS INHIBITORS OF HEATING AND LIPOLYSIS IN FLAXSEED

| Chemical | Effect on lipolysis | | Effect on heating | |
|---|---|---|---|---|
| | Concentration equivalent to 0.38% ethylene chlorhydrin (%)* | Relative effectiveness† | Concentration equivalent to 0.38% ethylene chlorhydrin (%)* | Relative effectiveness† |
| 1-Chloro-1-nitroethane | 0.42 | 0.9 | 0.42 | 0.9 |
| 8-Quinolinol | 0.42 | 0.9 | 0.42 | 0.9 |
| Orthovanillin | 0.42 | 0.9 | 0.42 | 0.9 |
| Phenol | 0.32 | 1.2 | 0.32 | 1.2 |
| Benzotrichloride | 0.45 | 0.85 | 0.45 | 0.85 |
| p-tert-Amyl phenol | 0.42 | 0.9 | 0.42 | 0.9 |
| Salicylaldehyde | 0.26 | 1.5 | 0.38 | 1.0 |
| 1,3-Dimethyl-4,6-bis(chloromethyl)benzene | 0.032 | 12.0 | ‡ | ‡ |
| Ethylene bromhydrin | 0.18 | 2.1 | 0.18 | 2.1 |
| Sodium cyanide | 0.042 | 9.0 | 0.017 | 23.0 |
| Propylene glycol dipropionate | 0.26 | 1.5 | 0.26 | 1.5 |
| Diethyl oxalate | >0.38 | <1.0 | 0.24 | 1.4 |
| Chloramine-T | <0.11 | >3.5 | ‡ | ‡ |

* Weight of inhibitor per weight of dry seed.

† Compared to 0.38 per cent ethylene chlorhydrin as 100.

‡ These compounds did not affect the heating of the seeds in the same manner as did ethylene chlorhydrin and were, therefore, not given a relative rating.

standard inhibitor were tested further at several other concentrations. Since there was no a priori reason to expect that the inhibitors would be equally effective on heating and lipolysis, the relative efficiency of each inhibitor was determined with respect to both effects. The comparative effectiveness of a series of chemicals is shown in Table 2.

The data reveal the fact that a wide variety of chemicals in fairly low concentrations are capable of inhibiting heating and lipolysis in moist flaxseed.[4] Although flaxseed was used in all of the experiments, a number of the inhibitors listed in Table 2 have also been used in treating moist cottonseed, rice, and grain sorghum and have been found to be equally effective. The low concentrations at which many of these compounds were effective as inhibitors of heating and lipolysis suggest their use in treating seeds on an industrial scale to improve their storage properties. Mill-scale experiments to determine the effectiveness of a number of these inhibitors are in progress.

No attempt is made here to interpret the results presented above in terms of the relation between chemical structure and biological activity of the compounds examined to date. It is probable that although their net effect, namely, inhibition of heating and lipolysis, is the same, the mechanism whereby this inhibition is achieved in the seed may differ for each inhibitor. It is necessary, therefore, to investigate their effect on the components of the enzyme systems involved in heating and lipolysis before any conclusions can be drawn regarding the relationship between chemical structure and biological activity.

Other investigators (e.g. *6*) have attributed the heating and deterioration of grain to the action of microorganisms associated with the grain. As has been pointed out in a previous publication (*4*), heating and deterioration can be equally well ascribed to the enzyme activity within the seeds themselves. It is also possible that the enzymes both in the seeds and in the microorganisms are responsible, perhaps in different degrees, for the observed biological activity of the seeds. It has not been possible to conclude definitely what relative role is played by the enzymes from the two sources.

The objective of this research has been to develop improved methods of storage of seeds intended for industrial or food utilization in which preservation of viability is not important. It is recognized that the development of improved conditions for the storage of planting stock constitutes an equally important problem, and it is hoped that some of the results of these investigations may eventually be applied in storing seeds without loss of viability.

### References


1. Altschul, A. M. *Oil Mill Gaz.*, 1944, **49**, 8, 13.
2. Altschul, A. M., Curet, M. E., Karon, M. L., Hall, C. M., and Smith, B. A. *Oil Mill Gaz.*, 1946, **50**, 9–13, 23.
3. Altschul, A. M., Karon, M. L., Kyame, L., and Caravella, M. *Oil & Soap*, 1943, **20**, 258–262.
4. Altschul, A. M., Karon, M. L., Kyame, L., and Hall, C. M. *Plant Physiol.*, 1946, **21**, 573–587.
5. Karon, M. L., and Altschul, A. M. *Plant Physiol.*, 1944, **19**, 310–325.
6. Milner, M., and Geddes, W. F. *Cereal Chem.*, 1946, **23**, 449–470.
7. Spiegelman, S., and Reiner, J. M. *J. gen. Physiol.*, 1945, **28**, 385–403.

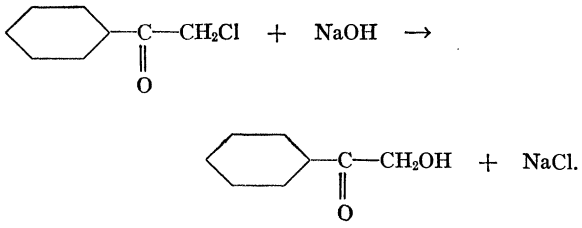

[4] In addition to the chemicals listed in Table 2, calcium propionate, glycol diacetate, diethyl malonate, and vinyl propionate have recently been found to act as inhibitors when used in concentrations equivalent to that of the ethylene chlorhydrin standard.

## Hydrolysis by Carbitolic Caustic


R. Macy

*Chemical Corps Technical Command, Edgewood Arsenal, Maryland*


Chloracetophenone (CN) has long been a popular lacrimator both for military and civilian uses. For example, it is a standard component of grenades employed by civilian police as well as by military forces. CN is characterized by rather high chemical stability, so that decontamination of an area covered by it can be accomplished by relatively few chemical treatments. One of the favorite points of attack on the molecule is the chlorine atom through hydrolysis by alkali:




Since CN is practically insoluble in water, the hydrolysis must be accomplished by employing the caustic in a solvent which will also dissolve the CN. Such a solvent is ethanol. A solution consisting of 5 per cent of sodium hydroxide and 95 per cent of ethanol will destroy a thin film of CN on a contaminated surface in a few minutes.

Because of the fire hazard introduced by ethanol in this decontaminating agent in certain military operations, a search was made for other mutual solvents for CN and sodium hydroxide. An added requirement for the solvent was that it should have good stability toward the alkali when the solution was stored. A large number of solvents were tested, and the search finally led to the following series of compounds:

| | | |
|---|---|---|
| (I) | $HOCH_2CH_2OH$ | Ethylene glycol |
| (II) | $HOCH_2CH_2OCH_2CH_3$ | Cellosolve |
| (III) | $HOCH_2CH_2OCH_2CH_2OH$ | Diethylene glycol |
| (IV) | $HOCH_2CH_2OCH_2CH_2OCH_2CH_3$ | Carbitol |
| (V) | $HOCH_2CH_2OCH_2CH_2OCH_2CH_2OH$ | Triethylene glycol |

As a result of these tests it was found that compound IV stood out quite sharply, not only for its solvent capacity for organic compounds but also for its stability toward alkali. The solubility of CN in dry carbitol at 22° C. was found to be 28.3 grams in 100 grams of solution, and in a mixture of 21 per cent of water and 79 per cent of carbitol (by weight) it was 9.3 grams in 100 grams of solution.

A solution containing, by weight, 5 per cent sodium hydroxide, 20 per cent water, and 75 per cent carbitol hydrolyzes CN quite rapidly when in thin film on an area. (In granular form CN dissolves rather slowly in carbitol, although it dissolves to a large extent, as already stated.) Higher concentrations of caustic can be used to give more rapid hydrolysis, but concentrations over 5 per cent may cause skin injuries unless personnel are well protected. The 5 per cent caustic solution in aqueous carbitol was found to be stable for at least two years in a sealed steel drum stored in the open without