stimulated in the hypothalamus to 0 to 11 percent of normal (Fig. 1). The response rates of two rats stimulated in the amygdala were depressed to 1 and 17 percent of normal. With the same dose, the response rates of six rats stimulated in the septum were depressed to a mean of 38 percent of normal (range, 0 to 77 percent). Three of the animals had scores of 50 percent or higher following administration of the drug. Thus, chlorpromazine appears to have selective effects similar to those of reserpine, but the effects are more variable.

Smaller doses of reserpine and chlorpromazine depressed the response rates of rats stimulated in the hypothalamus but rarely altered response rates in rats stimulated in the septal region.

Pentobarbital at doses of 10 mg/kg did not have similar selective depressant effects, although one aberrant animal showed a depressant effect. At doses of 15 mg/kg, marked motor depression made it difficult to assess the data; animals stimulated in the hypothalamus, however, have been seen to give high response rates even with this extreme dose.

Increasing sensitivity to reserpine on successive administrations at 1 or 0.5 mg/kg was also found in these experiments. This is illustrated in Fig. 1 by the greater depression caused by the second 1-mg and the second 0.5-mg dose for the rat stimulated in the hypothalamus.

From these preliminary studies it appears that, in the rat, the rate of self-stimulation through electrodes implanted deep in the brain may be used as a behavioral screening method to distinguish tranquilizing agents from other central nervous system depressants, and possibly also from each other. Reserpine and chlorpromazine, at doses without observable side effects, have been shown to depress selectively at certain brain sites, thus distinguishing them from pentobarbital, which has no selective effects. At doses of the tranquilizing agents large enough to produce gross changes in spontaneous motor activity, selectivity between animals stimulated in the septal region and the hypothalamus is no longer observed.

These observations are being extended by the use of other electrode placements and a wider dose range of these and other agents. Such techniques should lend insight into selective sites of action of tranquilizing agents. Studies now in progress relating primary drives to the various parts of the "rewarding" system may provide a basis for interpreting the differential drug effects.

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References and Notes

1. J. Olds and P. Milner, *J. Comp. Physiol. Psychol.* 47, 419 (1954); J. Olds, "Physiological mechanisms of reward," in *Nebraska Symposium on Motivation*, A. Maslow *et al.*, (Univ. of Nebraska Press, Lincoln, 1955), Chap. 3, pp. 73–139; J. Olds, *J. Comp. Physiol. Psychol.*, in press.
2. These studies were aided by contracts NR 110-402 [Nonr-233(33)] and NR 144-102 [Nonr-233(32)] between the Office of Naval Research, Department of the Navy, and the University of California, and by Eli Lilly and Company ("Neuropharmacology of anesthetics and excitant drugs").

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Heat Denaturation of Serum Albumin in Presence of Perfluorooctanoic Acid

Based on studies of the interaction between bovine serum albumin (BSA) and perfluorooctanoic acid (PF) ($C_7F_{15}COOH$) (*1*) and the initial observation that PF may prevent the heat coagulation of BSA (*2*) similar to the studies of Ballou *et al.* (*3*), an investigation was undertaken of the physical-chemical and immunochemical properties of BSA-PF complexes heated under controlled conditions (*4*).

Solutions of 0.33-percent BSA in the presence of varying amounts of PF in acetate buffers of 0.1-ionic strength at *p*H 5.44 or 5.72 were autoclaved at 105°C and 15-lb pressure in thin-walled, sealed, 10-ml ampoules for 20, 30, 40, 50, 60, and 120 minutes, respectively. The solutions were stored in a refrigerator for 1 week, after which it was observed that only those systems composed of a minimum of 266 moles of PF per mole of BSA remained clear. For the immunochemical reactions with calibrated rabbit anti-BSA sera, the various solutions were adjusted to *p*H 7 to 7.5 without appreciable change in protein concentration.

Sedimentation patterns were obtained at two rotor speeds—namely, 42,040 and 56,100 rev/min. Typical sedimentation constants, corrected for adiabatic expansion of the rotor, are assembeld in Table 1. From the variation of the sedimentation constants as a function of the gravitational field and of heating time, it may be concluded that heated BSA and PF form complexes of micellar nature. Because many types of micelles could exist —each with its own critical micelle concentration—one would expect that the area under each sedimenting peak would decrease with increasing sedimentation time and with change of concentration across the boundary. These area losses are different from those observed by Brand (*5*), inasmuch as they cannot be accounted for by the sedimentation of large aggregates at low speeds, and inasmuch as they become more pronounced as heating time is increased.

The reversibility of PF binding in heated samples was investigated by exhaustively dialyzing these solutions against 20 volumes of buffer and following their behavior at periodic intervals, both in the ultracentrifuge and by immunochemical analysis. The ultracentrifugation study (Table 1) indicated that micelles still remained, for area losses were observed in all dialyzed heated solutions. The *p*H of the dialyzates was raised to 7.4 in order to utilize electrostatic repulsion to dissociate complexes, but even this procedure did not reduce area losses, nor did we observe a component the sedimentation of which was equal to that of native BSA at the same concentration. When the *p*H of the dialyzates was low-

Table 1. Sedimentation constants of BSA-PF complexes. Area losses were observed in autoclaved samples. The numbers in parentheses represent average relative proportions (percentage) of total schlieren areas.

Experimental conditions	Sedimentation constants, $S_{20,w}$							
*p*H 5.44, mole ratio PF to BSA = 293/1								
Unheated, 56,100 rev/min	14.50	(84.3)	17.81	(15.7)				
Autoclaved 20 min, 56,100 rev/min	15.58	(37.5)	27.79	(50.0)	82.5	(12.5)		
Autoclaved 20 min, 42,040 rev/min	16.46	(36.6)	23.78	(63.4)				
*p*H 5.72, mole ratio = 468/1, 56,100 rev/min								
Unheated	10.47	(32.5)	13.95	(67.5)				
Autoclaved 30 min	7.35	(9.0)	10.98	(50.4)	14.74	(32.3)	20.82	(8.3)
Autoclaved 60 min	10.69	(57.6)	14.64	(42.4)				
*p*H 5.72, mole ratio = 366/1, 56,100 rev/min								
Autoclaved 120 min	11.13	(39.2)	15.14	(45.9)	18.95	(14.9)		
Autoclaved 120 min, dialyzed 3 times, *p*H 5.72	5.76	(44.4)	14.60	(55.6)				
Autoclaved 120 min, dialyzed 5 times, *p*H 7.41	4.55	(67.2)	10.72	(32.8)				
BSA control	4.05	(100)						

Table 2. Immunochemical reaction of autoclaved BSA-PF complexes, with rabbit anti-BSA serum.

*p*H at heating time	mole ratio	heating time (min)	Denaturation (%)
5.72	366/1	120	100
5.72	293/1	20	73
5.44	293/1	20	73
* 5.72	293/1	20	87
† 5.44	293/1	20	67

* Dialyzed five times against *p*H 7.5 buffer.
† Dialyzed against buffer of *p*H 4.9, precipitate removed, supernatant redialyzed against buffer of pH 7.5.

ered to 5.2, a sizable precipitate appeared. This property, as well as the general appearance of the exhaustively dialyzed solutions in the ultracentrifuge, resembled observations reported on heat-denatured BSA (*6*).

The aggregation phenomenon in unheated samples is strikingly different from that in autoclaved ones. No physical-chemical or immunochemical evidence of denaturation is observed provided that heating is eliminated. Even in the presence of large amounts of PF, where aggregation is very pronounced, the following properties of BSA remain unchanged: partial specific volume at 25.0 ± 0.03°C at 0.734, intrinsic viscosity at 0.0414. Under the same conditions, the partial specific volume of PF was 0.401 and its intrinsic viscosity 0.0108. This might indicate that the over-all hydrodynamic shape of the unheated aggregates is not markedly different from that of the native BSA molecule.

A mechanism of BSA-PF complex formation at low temperatures was previously proposed (*7*) by postulating that the fluorine atoms interact with protein hydrogen bonds. This interaction could be reversible as long as the water layer surrounding the protein remains essentially undisturbed. At high temperatures, where the configuration is known to be disrupted, it is conceivable that PF molecules could penetrate the water layer, form some "direct" hydrogen bonds, and thus solubilize the denatured protein near its isoelectric point (*p*H 5.2). Exhaustive dialysis at that *p*H, however, precipitates the heat-denatured protein.

Immunochemical studies with BSA-PF complexes corroborated the physical-chemical findings that PF does not protect BSA from heat denaturation. Although quantitative immunochemical techniques were employed in these studies, the results obtained can only be interpreted qualitatively. Results obtained in the region of antibody excess through the equivalence zone indicated that significant changes had occurred in BSA. The figures in the last column of Table 2 refer to the amount of the protein relative to native BSA which did not precipitate with anti-BSA. This does not imply that the heated complex that did precipitate with anti-BSA was completely unaltered, as shown by the following. (i) If the BSA-PF complexes are not fractionated by differential dialysis, the maximum amount of total nitrogen could never be precipitated. (ii) Even after dialysis at *p*H 5.6 and 4.9, which led to the removal of appreciable amounts of insoluble (denatured) material, the soluble protein did not react like native BSA. In some cases its behavior resembled that of an aggregated molecule as evidenced by a broad equivalence zone.

From the results presented here (*8*) it would appear that BSA cannot be protected against heat denaturation at 175°C. Furthermore, from viscosity and partial specific volume studies alone, no deduction can be made concerning the interaction of unheated BSA-PF complexes. A serious discrepancy therefore exists between our findings and results previously published (*9*).

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References and Notes

1. H. B. Klevens and E. Ellenbogen, *Discussions Faraday Soc.* 18, 277 (1954).
2. H. B. Klevens, private communication.
3. G. A. Ballou *et al., J. Biol. Chem.* 153, 589 (1944).
4. Presented in part at the Third International Congress of Biochemistry (1955) (*Résumé de Comm.*, p. 12) and at the 128th National Meeting of the American Chemical Society (1955) (*Abstr.*, p. 24C). This investigation was supported in part by a grant (F-4014C) from the National Institutes of Health, U.S. Public Health Service, to Eric Ellenbogen and by contract No. 124–229 NONR1077(00) (P.H.M.) between the Office of Naval Research and the University of Pittsburgh.
5. B. P. Brand, *Discussions Faraday Soc.* 18, 312 (1954).
6. S. Ehrenpreis and P. H. Mauer, in preparation.
7. E. Ellenbogen, *Discussions Faraday Soc.* 18, 309 (1954).
8. A detailed report of this investigation is in preparation.
9. H. B. Klevens, *Nature* 176, 879 (1955).

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On Respiratory Impairment in Cancer Cells

Some years ago Otto Warburg (*1*) enunciated a theory of cancer which, briefly summarized, proposed that cancer originates when a nonneoplastic cell adopts an anaerobic metabolism as a means of survival after injury to its respiratory system. According to Warburg, the tumor is initiated by a damaged respiration, which persists as a characteristic feature of the neoplastic condition. In a recent paper, entitled "On the origin of cancer cells," originally published in German (*2*) and then translated into English (*3*), Warburg reiterates this hypothesis and claims further support for it on the basis of experiments with ascites tumor cells. I recognize the great debt that biochemists owe this illustrious investigator and regret the necessity of taking issue with his basic biochemical premise, namely, that cancer cells have an impaired respiration.

In a comprehensive review of this subject in 1939, Burk (*4*) first pointed out the essentially fallacious reasoning behind this hypothesis. More recently, Schmidt (*5*) and I (*6*) reviewed this topic independently in the light of modern findings and concluded similarly that there is no sound experimental basis for the belief that oxidative metabolism in tumors is impaired. It is recognized by all, including Warburg, that despite their high glycolysis, oxygen consumption is not quantitatively diminished; by and large, a representative group of tumors absorb oxygen about as rapidly as a comparable group of nonneoplastic tissues (see, for example, Burk's extensive tables, *4*). An early statement by Warburg, illustrative of his views concerning the relationship between the high aerobic and anaerobic glycolysis of tumor cells and their oxygen consumption, is the following (*1*, pp. 139–141).

"We determined the Meyerhof quotient for carcinoma tissue, lactic acid bacteria, embryonic tissue and a number of other glycolyzing tissues, and as a rule obtained the same mean values as Meyerhof. As a rule 1 mol. of breathed oxygen, just as in muscle, causes the disappearance of 1–2 mol. lactic acid. This result . . . proves that the influence of the respiration on the cleavage metabolism in the carcinoma-cell is normal. . . . Although in the tumor every oxygen molecule breathed is just as effective as in muscle—the Meyerhof quotient is equal in the two cases—yet the respiration does not cause the glycolysis to disappear. The respiration of the carcinoma tissue is too small in comparison with its glycolytic power."

Thus, according to Warburg, the Meyerhof quotient (a quantitative expression of the Pasteur effect) is normal in carcinoma, and oxygen consumption is also not quantitatively diminished; but respiration is disturbed, because glycolysis persists in oxygen. As I pointed out earlier (*6*, p. 276), I believe it would be more accurate to state that anaerobic glycolysis is so high in tumors that a normal respiration and a normal Pasteur effect are incapable of eliminating it.

Although Warburg still states categori-