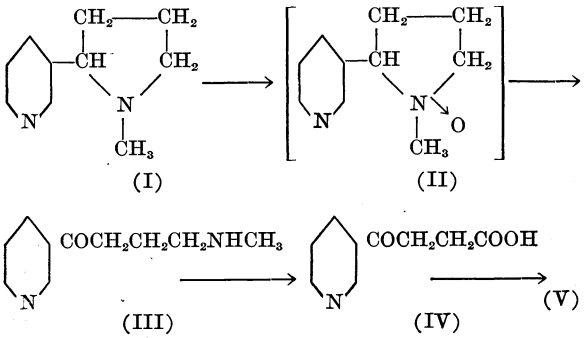
formula of $C_9H_9O_3N$ and may be derived from pseudo oxynicotine by oxidative activities of the microorganism. This acid seems to be 3-succinoyl pyridine, and its constitution is now being studied by synthesis from nicotinic acid.

From the above-mentioned results we may now assume the mechanism of the decomposition of nicotine by the microorganism as follows:



Substance VI has almost the same characteristics as the compound obtained by Bucherer. (Anal: Found: C, 55.40; H, 4.65; N, 7.68.) Substance V, which may be produced through further decomposition from IV, is not extracted with ether from the acidic or alkaline solution, but from the acidified solution substance V is precipitated as picrate, mp 316° C, when recrystallized from 60% methanol (Anal: Found: C, 27.29; H, 1.34; N, 16.77.)

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Influence of Age of Serum on Microbiological Response[1]

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During investigations in this laboratory involving microbiological assays of blood from patients with malignant neoplastic diseases, it has been observed that storage of serum significantly influences the degree of response obtained with certain lactic acid organisms.

The basal medium contained 2% glucose and salts as employed by Dunn *et al.* (*1*), but 0.03%–0.05% Difco yeast extract was added in place of the growth factors and amino acids used by these workers. In the absence of yeast extract, significant growth response to serum did not occur. With a yeast extract concentration of 0.03%–0.05%, satisfactory responses to serum were obtained, with minimal growth in the control medium without serum. The nature of the component(s) of serum responsible for the observed stimulation of growth of microorganisms is unknown and, in fact, was not a primary concern of the studies reported here.

Double-strength medium was brought to boiling, filtered, and autoclaved for 15–20 min at 15 psi. Cultures were carried on Difco micro inoculum agar and were usually transferred twice in micro inoculum broth before use. Cells were washed once with distilled water, and 1–2 drops of a 1:100 dilution were used as inoculum. Incubation was at 37° C. Sera were obtained from prisoners at the state penitentiary in Huntsville, Texas, and from employees and patients in this hospital. All sera were presumably fasting. Hemolyzed sera were discarded, since it was noted that such sera yielded abnormally high responses. Growth was estimated, generally from triplicate tubes, in terms of optical density (log L_0/L) calculated from Coleman spectrophotometer readings at 590 μ. Serum concentrations employed ranged up to 25%.

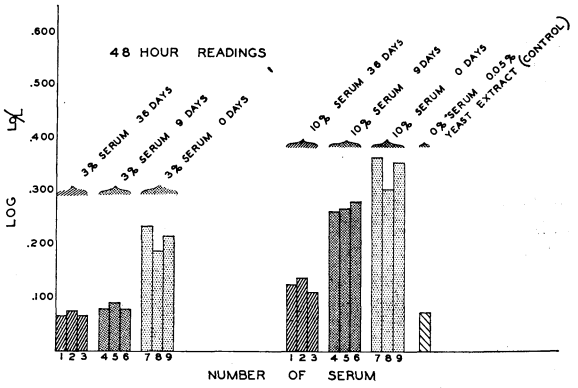

FIG. 1. Variation in response of *L. casei* Texas with age of normal sera.

Three groups of 3 sera each stored at 4° C for 0, 9, and 36 days, respectively, were tested simultaneously at two concentrations with *Lactobacillus casei* Texas (Fig. 1). Two control experiments, one with 10 fresh normal sera and the other with 5 fresh normal sera and 5 fresh sera from cancer patients, failed to show variations approaching the magnitude of those shown in Fig. 1. Responses to sera from patients with malignant neoplastic disease were similar to those obtained with normal sera.

Six pools of sera collected from five volunteers at varying times over a period of a month were tested simultaneously at a concentration of 10% with five organisms. A marked decrease in response of *L. casei* Texas occurred with increasing age of serum, whereas responses of *Lactobacillus brevis* ATCC 8287 and

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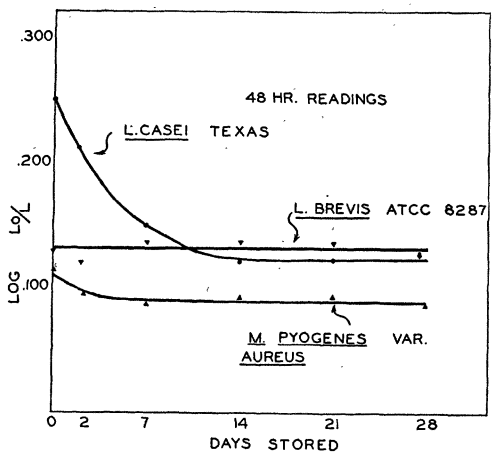

FIG. 2. Response of three assay organisms to serum pools stored for varying times at 4° C and tested simultaneously.

Micrococcus pyogenes var. *aureus* were relatively constant (Fig. 2). In data not shown, responses of two strains of *Lactobacillus arabinosus* (ATCC 8014 and MC-Oklahoma) showed significant decreases with storage, although the effects were somewhat less marked than with *L. casei*.

In a survey of stock cultures, a carefully standardized procedure was used, with one large serum pool stored at 4° C and tested at various times after collection. As shown in Table 1, responses of eight organisms were affected by age of the serum. On the other hand, no effect was observed with two strains of *Leuconostoc dextranicum* (ATCC 8358 and ATCC 8086), *Leuconostoc mesenteroides* ATCC 8293, *L. brevis* ATCC 8287, *Lactobacillus fermenti* Texas, and *M. pyogenes* var. *aureus*. Responses of *L. casei* ATCC 9595 and *Streptococcus faecalis* ATCC 9790 were subsequently shown to be markedly affected by age of serum, but *L. mesenteroides* ATCC 8042 and *Lactobacillus pentoaceticus* ATCC 367 gave equivocal data on repeated experiments.

The mechanism of development of decreased microbiological response on storage of serum is unknown. It is pertinent to note, however, the following findings, obtained principally with the *L. casei* strains at serum concentrations of 10%: (*a*) In studies at 37° C, room temperature, 4° C, and −15° C, *rate* of development of lessened response decreased with decreasing temperature of storage. Similar results were obtained with *Str. faecalis* ATCC 9790 and *L. arabinosus* ATCC 8014. (*b*) The *rate* of development of lessened response in sera from different individuals exhibited rather wide variation. The presence of malignant neoplastic disease caused no distinctive alteration in response patterns. (*c*) Since sera readily "aged" in an atmosphere of natural gas (approx composition:[2] CH_4, 94%; C_2H_6—C_5H_{12}, 5.1%; CO_2, 1.3%; N_2, 0.6%), the phenomenon would appear to be independent of oxygen tension. (*d*) The addition of 0.1% soluble starch did not affect responses of eight sensitive species tested. Since starch has been reported to overcome the toxicity of C_{18} unsaturated fatty acids for at least some bacteria (*2, 3*), an accumulation of such fatty acids from serum lipase activity would appear unlikely. (*e*) Responses to varying proportions of aged serum and fresh serum at a total serum concentration of 10% were more indicative of decrease in nutritive value than of appearance of toxicity during storage of serum. (*f*) Heating fresh serum at 60° C for 30 min had no apparent effect on the rate of development of lessened response on storage at 37° C, although heating did markedly increase the degree of response. Heating stored serum resulted in some increase in response, which did not decrease on further storage. (*g*) Microbiological assays[3] of 17 free amino acids and comparison of paper partition chromatograms[3] of tungstic acid filtrates of fresh and stored pooled sera indicated that the stored serum contained less cystine and more methionine, phenylalanine, and glutamic acid than the fresh serum. However, supplementation with any one of these four amino acids or with a mixture of 16 amino acids did not significantly affect responses to fresh or aged sera.

TABLE 1

72-HR RESPONSES (LOG L_0/L) TO POOLED SERUM AT 10% CONCENTRATION

Organism	Serum stored at 4° C (days)			Basal medium
	0	6	20	
Streptococcus faecalis ATCC 8043	0.082	0.036	0.029	0.032
Str. faecalis Texas	.112	.059	.060	.052
Str. lactis Texas	.109	.066	.070	.053
Leuconostoc mesenteroides Texas	.103	.053	.060	.052
L. citrovorum ATCC 8082	.120	.065	.062	.075
Lactobacillus arabinosus MC-Oklahoma	.207	.131	.117	.060
L. arabinosus ATCC 8014	.184	.130	.110	.063
L. casei Texas	0.280	0.159	0.138	0.035

As stated above, the nature of the substance(s) responsible for the growth stimulation observed with serum was not a primary concern of the present investigation, although we realize that this problem merits attention. The point to be emphasized is that the observed significant effects of storage of serum on microbiological response could conceivably be of importance in many microbiological assays, particularly in view of the probability of storing pooled control serum in the refrigerator until needed. Such stored control serum assayed simultaneously with fresh test sera could yield spurious data and result in erroneous conclusions with regard to the effect of a given factor or condition. In fact, the phenomenon of decrease in growth response on storage of serum was discovered on close scrutiny of an assay procedure which at first sight appeared to show promise in the detection of cancer. Differences obtained be-

[2] Courtesy United Gas Corporation, Houston, Tex.

[3] The authors wish to express their appreciation to Robert Fuerst, of the Department of Biochemistry, who performed these analyses.

tween normal sera and sera from patients with cancer were, of course, attributable to the fact that sera from the cancer cases were assayed shortly after venipuncture and compared with control serum that had been stored in the refrigerator.

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A Study of the X-Irradiation Protection Afforded by Cobalt

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It is generally believed that hemopoiesis is the key to recovery after irradiation injury. Even if this fact does not assure survival of irradiated animals (*1*), it seemed of interest to study the influence of polycythemia-producing substances on irradiation effects. Such a substance is cobalt, which is known to produce polycythemia. The mechanism of this cobalt polycythemia is unknown. It is assumed that cobalt interferes with cellular respiration (*2*), a process of decisive importance again for the magnitude of the irradiation damage. With these facts in mind, investigations on the influence of cobalt in total body irradiation effects were undertaken.

Approximately 400 female Swiss Albino mice (25 ± 1 g) were used. They were divided into four groups for each experiment: group 1 on Purina stock chow diet, group 2 on cobalt diet, group 3 on Purina stock chow diet plus irradiation, group 4 on cobalt diet plus irradiation. The cobalt diet was prepared by immersing the Purina chow pellets in a 2% cobalt chloride solution ($CoCl_2 + 6\ H_2O$) for a 2-min period and then allowing them to dry. The mice were kept on this diet 5 and 8 days before irradiation and for about 15 days after irradiation. Food and water were given *ad lib.*

The mice were irradiated in groups of 10, each group consisting of 5 on Purina chow and 5 on the cobalt diet. The irradiations were done with a Kelley-Koett deep therapy x-ray unit, operated at 200 kv, 6 ma, inherent filtration equiv 0.25 mm Cu, 1 mm Al plus 0.5 mm Cu added, 30 cm target distance, 48 r/min in air; a total of 720 r/air. The mice were observed for 30 days after irradiation, and the mortality rate was recorded every 24 hr.

The results obtained with animals receiving cobalt food for 5 and 8 days prior to irradiation are presented in Figs. 1 and 2. The animals kept on cobalt diet before irradiation show in both cases a significant increase in their resistance against irradiation in comparison to the irradiated groups on Purina chow only.

The general appearance of the cobalt-fed irradiated

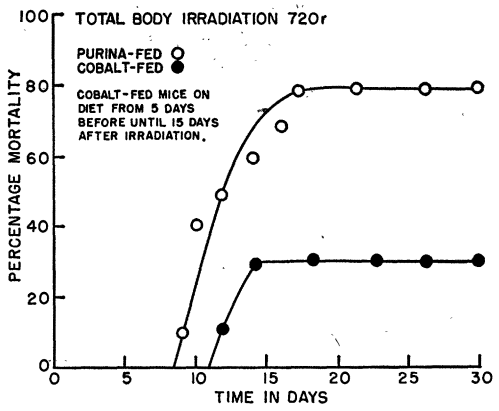

FIG. 1. Mortality rates.

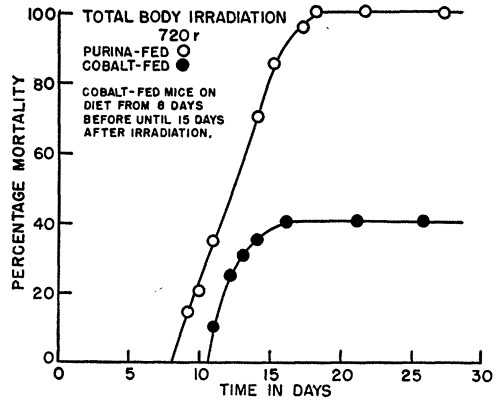

FIG. 2. Mortality rates.

animals was quite similar to that of the nonirradiated animals. They exhibited smooth fur and normal vitality, whereas the irradiated animals on normal food and the few animals that died in the cobalt-fed irradiated group showed all the signs of heavy irradiation damage. To obtain the beneficial effect the weight of the animals, the preparation of the cobalt food, and ease of accessibility to food and water are of decisive importance.

The protective effect exhibited by cobalt lends itself to discussion and comparison with present irradiation protection investigations.

An oxygen deficiency increases the resistance against irradiation damage (*3, 4*). Compounds such as cysteine and glutathione counteract irradiation effects on sulfhydryl enzymes (*1, 5*). The proper support of the hematopoietic system stimulates and speeds recovery (*6, 7*). In cobalt administration experiments, similar processes are going on. According to Barron and Orten (*8, 9*), cobalt interferes with cellular respiration and produces anoxia. According to Burk *et al.* (*10*), cobalt may block sulfhydryl groups and perhaps other groups necessary for tissue metabolism. According to Orten (*9*), cobalt produces a strong polycythemia, indicating an active stimulus to the hematopoietic system.

Which of these mechanisms is decisive in the ob-