Tissue Isotope Clearance.
II. Reduction of Clearance by Pentobarbital Infusion[1] (35791)

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Kety (1) demonstrated that local depression of circulation (by epinephrine or a tourniquet) caused a reduction of $^{24}Na^{+}$ clearance from muscle injection sites. Conversely, stimulation of the circulation by hyperemia or vasodilators increased the clearance rate. These observations have been confirmed by numerous investigators using various labeled substances (2, 3).

The Kety method involves detecting clearance of a substance from injection sites by external monitoring. Since the geometrical relationship of detector to injection site must be maintained constant, the organ being studied must be immobilized throughout the clearance. In experimental animals, immobility is accomplished through anesthesia, usually by a barbiturate.

The barbiturates cause progressive depression of the circulation with increasing dose (4, 5). In spite of this important relationship of anesthetic depth to the circulation, no reports appear in the literature relating depth of barbiturate anesthesia to the clearance rate of injected isotopes.

The following experiments demonstrate that increasing depth of pentobarbital anesthesia progressively diminishes tissue isotope clearance rate and that following circulatory failure and death, the clearance is completely arrested.

Methods. Five female rats, averaging 348 g in weight, were anesthetized with a solution of pentobarbital sodium (40 mg/kg) by ip injection. The pentobarbital solution was prepared each day by adding Na pentobarbital to an isosmotic phosphate–bicarbonate buffer solution,[2] which was then titrated to pH 7.4. When the withdrawal reflex was lost, the carotid artery was cannulated for measurement of blood pressure and the jugular vein was cannulated for infusion of solutions. The treachea was cannulated to allow free breathing of room air and the rat was placed dorsal side up. Rectal temperature was monitored by a Yellow Springs thermistor and the temperature limits of the rats were controlled by radiant heat between 100 and 102°F.

Complete methods of preparing the isotope solutions, performing the injections, and analyzing the clearance rates were previously reported (6). Clearances are here reported as initial clearance rate, C_i.[3]

Control isotope clearances were determined during infusion of the phosphate–bicarbonate buffer into the jugular vein (0.02 ml/min for 10 min by means of a Harvard pump). A subcutaneous injection of 0.1 ml of the buffered solution containing ^{131}I-

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[2] The phosphate–bicarbonate buffer was 0.005 *M* with respect to phosphate, 0.08 *M* with respect to bicarbonate and 0.08 *M* with respect to NaCl.

[3] C_i, the initial clearance rate, is the slope of the initial portion (from 1.5 to 6.5 min) of the clearance function obtained by plotting cpm vs time and is an approximation of Kety's k constant ($\times 100$) from the equation $A_t = A_0 e^{-kt}$; where A = amount of isotope present at times, t and 0. The initial clearance rate (C_i) is obtained from the following formula:

$$C_i = \frac{100\,[1-(At_2/At_1)]}{t_2-t_1},$$

where C_i = % cleared/min, A = cpm at times t_1 or t_2.

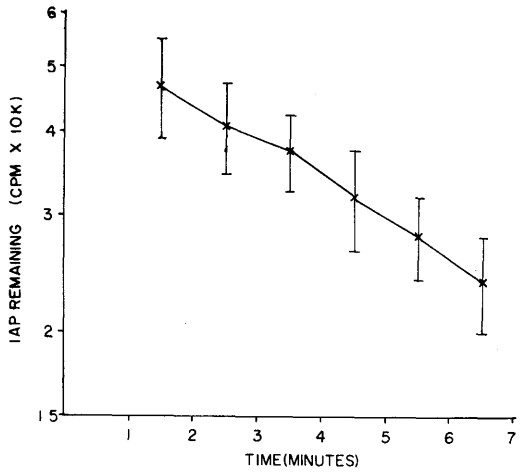

FIG. 1. Mean IAP remaining during control clearances from subcutaneous tissues of rats ($N = 5$) (calculated C_i from this curve is 9.4% ± 0.83).

4-iodoantipyrine (IAP) was performed in the midline of the dorsal lumbosacral region (previously shaved) and the clearance rate was determined by external monitoring of isotope disappearance by means of a Nuclear Chicago surgical scintillation detector model DS8-1. After determination of this control clearance, the infusion solution was changed to pentobarbital (prepared in the manner described above). When a steady blood pressure was established at this initial barbiturate infusion rate, a second subcutaneous IAP clearance was performed at a site near the control site. The degree of barbiturate depression was then increased by doubling the infusion rate (or by using a more concentration pentobarbital solution) during two or three infusion periods of 10–20 min each. During each pentobarbital infusion period another isotope clearance was performed in the midline near the control site.

The maximum rate that solution was infused in any one rat was 0.206 ml/min. The maximum volume of solution infused into a rat was 3.96 ml or 8.73 ml/kg.

Results. For the five rats, the mean control activity of the remaining IAP is plotted against time (Fig. 1). Analysis of this curve gives a C_i for control injections (during phosphate buffer infusion 0.02 ml/min for 8 min) of 9.4% ± 0.83 (SEM). In all five rats, there was a decrease in clearance rate after the infusion of pentobarbital was started and further decreases in clearance as pentobarbital infusion rates were increased (see Fig. 2, BP, C_i). Within 20 min after death, the clearance rate dropped to zero in all rats of the series. The latter observation was confirmed in a separate series of six male rats (C_i before death = 10.7 ± 1.27, after death = 0).

An inverse relationship between blood pressure and pentobarbital infusion rate (PIR) was also demonstrated (Fig. 2), the blood pressure falling as the PIR is increased. Also, there appears to be a direct proportionality between blood pressure and the isotope clearance rate (Fig. 2), since the IAP clearances (C_i) diminishes with falling blood pressure.

Discussion. From the experiments here presented, it can be concluded that the clearance rate of an injected substance depends upon the degree of depression by barbiturates. There appears to be a direct relationship between increased pentobarbital infusion rate, decreasing blood pressure, and decreasing tissue isotope clearance. Since pentobarbital causes circulatory depression (4, 5) which in turn decreases peripheral blood flow, it

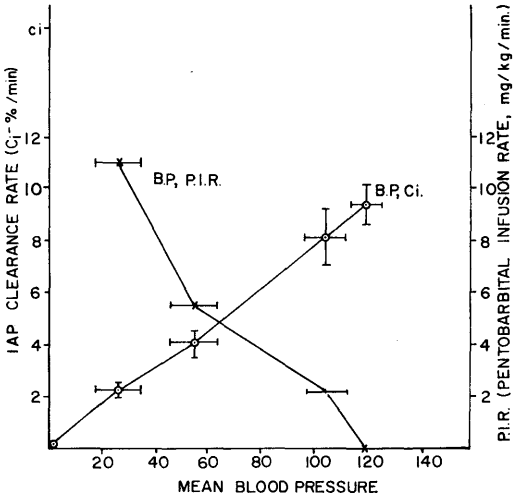

FIG. 2. Relationship of the blood pressure fall, pentobarbital infusion rate (PIR) and isotope clearance rate (C_i) of IAP in rats ($N = 5$). Mean blood pressure was calculated by averaging the mean blood pressure at the beginning and at the end of the clearance period.

may be postulated that the cause of the decreasing isotope clearance is circulatory depression. Kety (1) postulated that isotope clearance is directly dependent upon capillary efficiency. This postulate has been well documented by many other investigators (2, 3, 7), as well as by the experiments reported here.

In these experiments there was no attempt to control respiration and indeed pentobarbital-induced respiratory depression was the probable cause of death. No attempt was made to sort out the relative effects of circulatory and respiratory depression on the isotope clearance rate. However, in recent experiments performed in our laboratory using well-ventilated dogs (data not shown), the effects of pentobarbital infusion upon subcutaneous isotope clearance was generally similar to that seen in the rats. Thus, pentobarbital depression of subcutaneous isotope clearance appears to be related to circulatory depression caused by the barbiturate, rather than respiratory depression.

The observation that no clearance occurs after death confirms that isotope clearance is dependent upon a functioning circulation and that diffusion of the isotope into surrounding tissues appears to play no role in disappearance of the isotope. This observation also supports Kety's hypothesis that clearance of an injected freely diffusible substance depends upon blood flow.

Since the clearance is a measure of "effectiveness of local circulation in removing solutes which resemble the tracer" (7), the Kety method could be used to evaluate microcirculatory effects of barbiturates, as well as of other drugs. The evaluation of microcirculatory efficiency by this method following increasing barbiturate dosage has not been reported in the past.

Summary. The clearance of a substance from a subcutaneous injection site is influenced by the depth of pentobarbital anesthesia. ^{131}I-4-iodoantipyrine showed an initial clearance rate of 9.4%/min after subcutaneous injection in anesthetized control female rats. Pentobarbital Na was infused at progressively increasing rates, resulting in a fall in blood pressure proportionate to the dose of barbiturate. With the fall in blood pressure a progressive fall in initial clearance rate of the injected isotope was also noted; after death no further clearance occurred. The data supports Kety's hypothesis that the clearance rate of a rapidly diffusible substance depends upon circulatory efficiency.

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