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Transfer in Handedness in the Rat Induced by Acetylcholine and Forced Practice

Charles A. Baker

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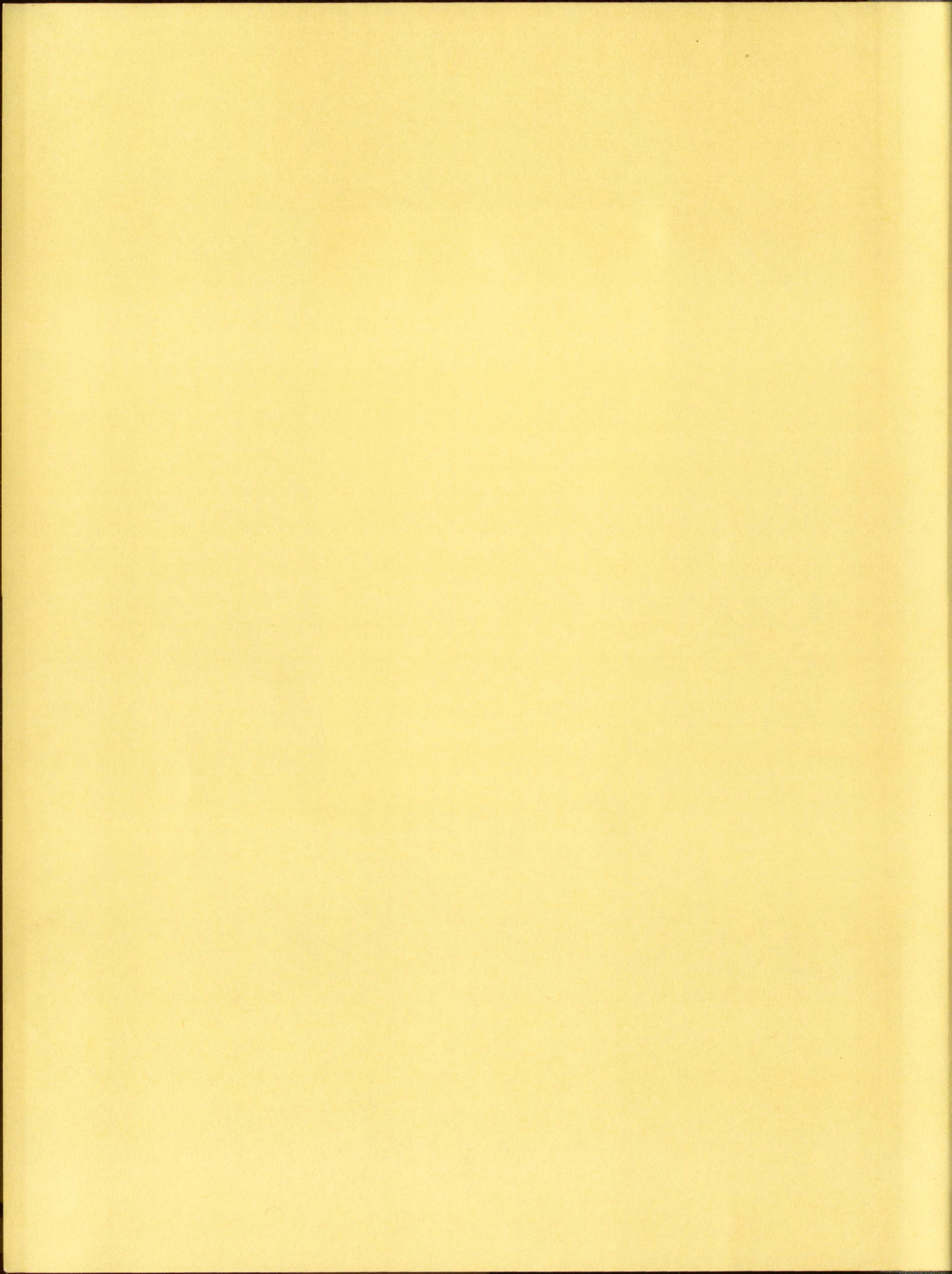
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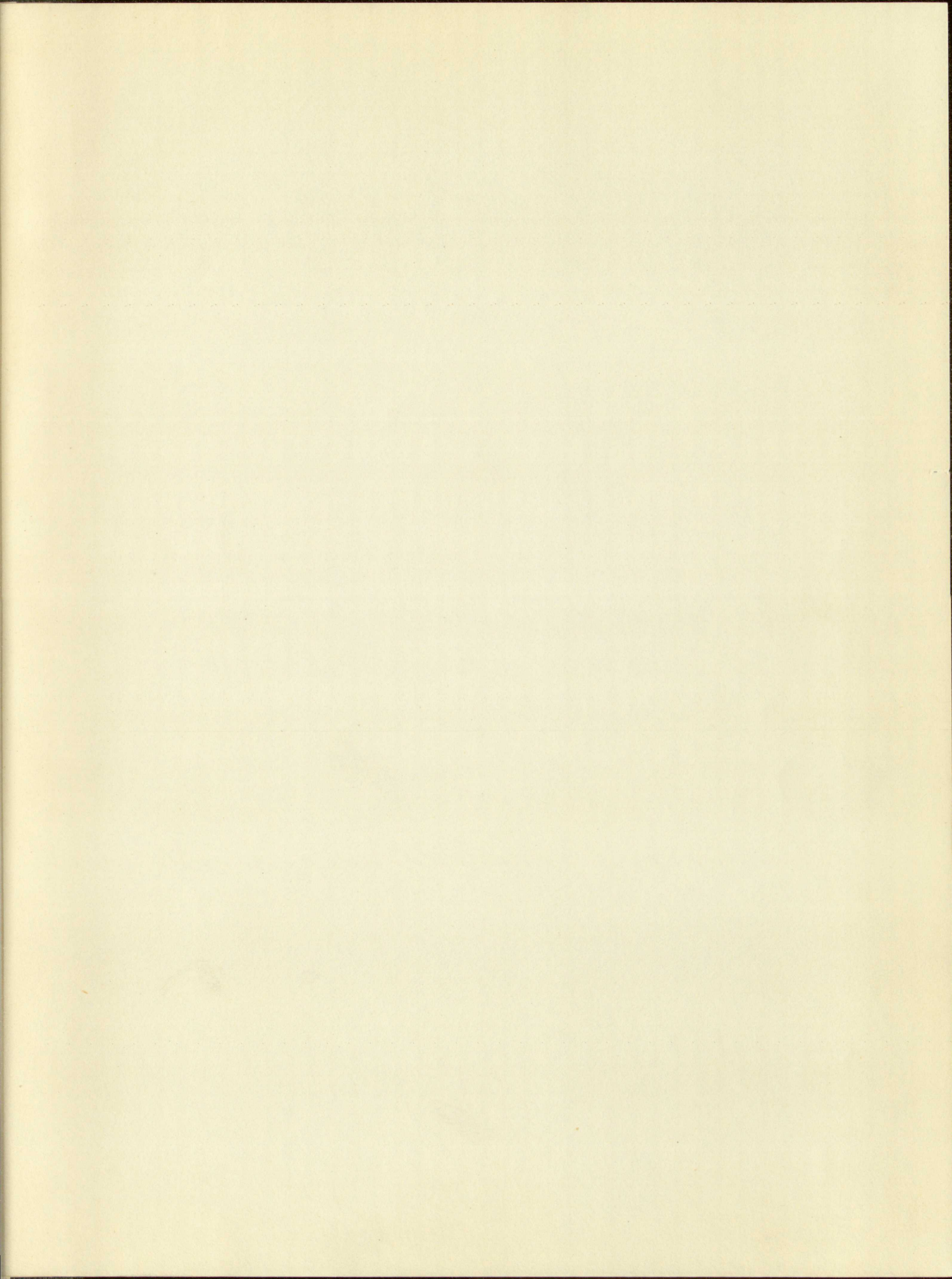
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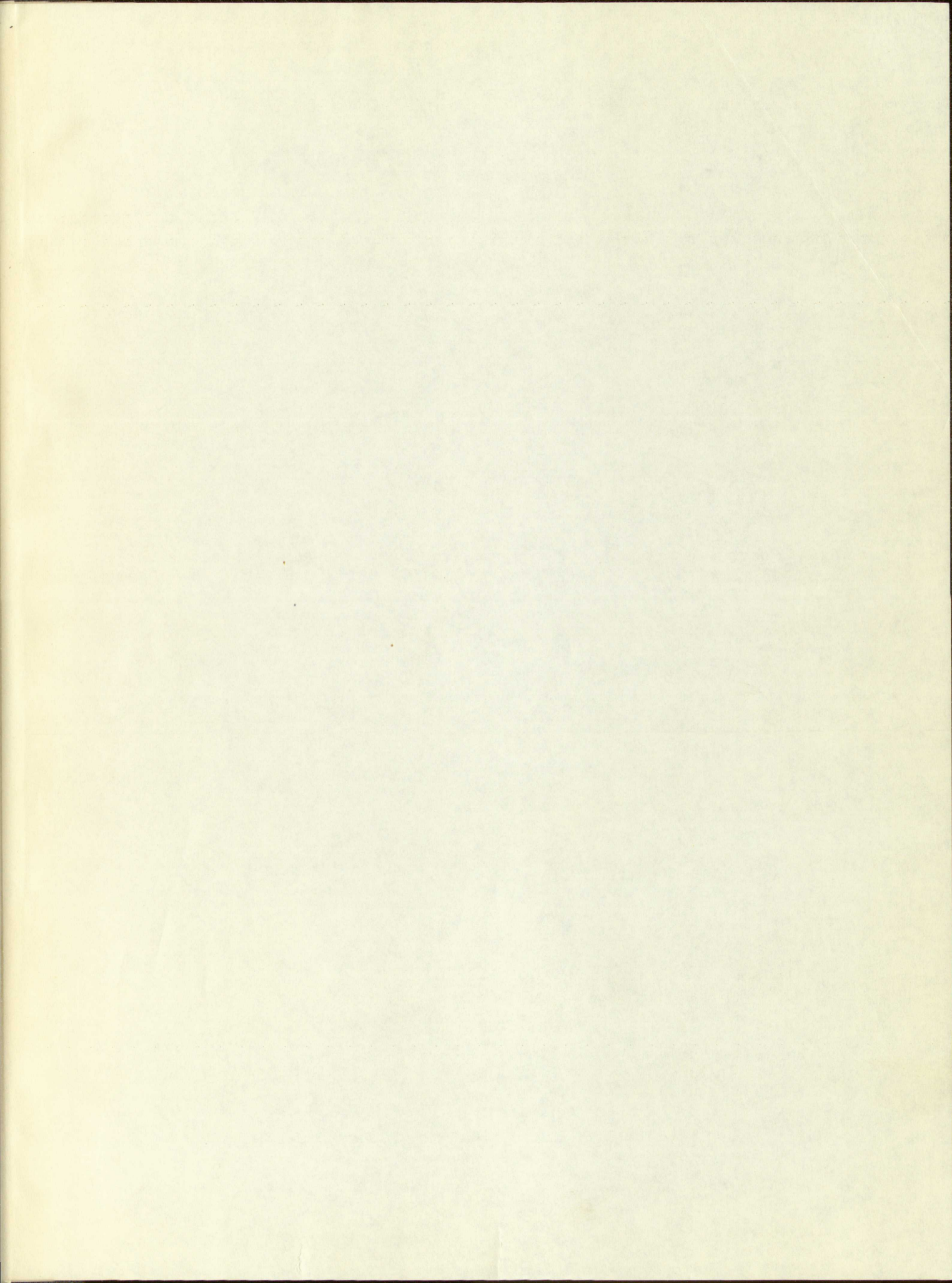
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TRANSFER IN HANDEDNESS IN THE RAT
INDUCED BY ACETYLCHOLINE AND FORCED PRACTICE

By

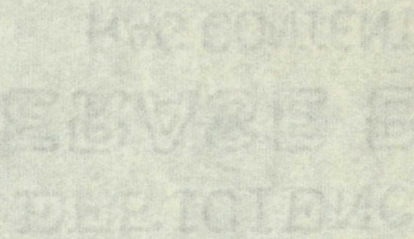
Charles A. Baker

A Thesis

In Partial Fulfillment of the
Requirements for the Degree of
Master of Arts in Psychology

The University of New Mexico

1950





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MASTER OF ARTS

E. J. Casteller

DEAN

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DATE

Thesis committee

Geo. W. Peterson

CHAIRMAN

Robert F. Zetter

Howard Abel

This thesis, directed and approved by the candidate's com-
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Table I

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EXERCISES

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Introduction and Statement of the Problem. Transfer in handedness in the rat has been successfully produced by two methods: by damaging functional tissue regulating this activity; and by forced practice with the non-preferred limb.

Peterson conducted a series of systematic studies enabling him to localize the cerebral region controlling handedness. ^{1, 2, 3} Extirpation of a small area in the contralateral frontal lobe will produce transfer, while extirpation of any other part of the cortex is ineffectual in producing transfer in handedness. He describes this locus more precisely as being approximately 250 microns posterior to the genu of the corpus callosum, directly over the dorsal convexity of the caudate nucleus, and below layer III of the cortex, probably in layer V.

The effect of forced reaching on handedness was also investigated by Peterson. ⁴ This work had been done

¹ Peterson, G. M., "Mechanisms of Handedness in the Rat," Comparative Psychology Monographs, 1934, 9, 1-67.

² Peterson, G. M., and Fracarol, J. C., "The Relative Influence of the Locus and Mass of Destruction Upon the Control of Handedness by the Cerebral Cortex," Journal of Comparative Neurology, 1938, 68, 173-190.

³ Peterson, G. M., and Chaplin, J. C., "Extrapyramidal Mechanisms in Handedness in the Rat," Journal of Comparative Psychology, 1942, 33, 343-36.

⁴ Peterson, G. M., op. cit., 1-67.

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- 2 Peterson, G. M., and Trenchard, J. G., "The Relative Influence of the Locus and Mass of Destruction Upon the Control of Handedness by the Cerebral Cortex," Journal of Comparative Neurology, 1938, 68, 173-190.
- 3 Peterson, G. M., and Gaglian, J. O., "Experimental-
dal Mechanisms in Handedness in the Rat," Journal of Com-
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- 4 Peterson, G. M., op. cit., 1-67.

on rats which had been subjected to cerebral injury. His method was to force the rat to reach with the non-preferred hand by binding the preferred hand with adhesive tape. In a later study Wentworth produced forced reaching by means of an offset dish.⁵ In this situation the dish is adjacent to a partition so that it is inconvenient to reach with the preferred hand. To obtain the food the rat is required to use the non-preferred hand. Peterson used this method in a systematic study of the effects of various amounts of forced reaching upon handedness.⁶ He found that the greater number of forced reaches resulted in greater transfer of handedness.

Although these two methods effect handedness they do not contribute directly to any knowledge of how the cerebral mechanisms function. In an effort to discover the nature of such cerebral activity Peterson selected a drug as a possible means to facilitate transfer.⁷ The drug chosen was acetylcholine because the findings

⁵ Wentworth, L. L., "Some Factors Determining Handedness in the White Rat," Genetic Psychology Monograph, 1942, 26, 55-117.

⁶ Peterson, G. M., "Transfers in Handedness in the Rat from Forced Practice," Journal of Comparative Neurology, In Print.

⁷ Peterson, G. M., "Changes in Handedness in the Rat by Local Application of Acetylcholine to the Cerebral Cortex," Journal of Comparative and Physiological Psychology, 1949, 42, 404-412.

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⁷ Peterson, G. M., "Changes in Handedness in the Rat by Local Application of Acetylcholine to the Cerebral Cortex," Journal of Comparative and Physiological Psychology, 1950, 43, 404-412.

of neurophysiologists indicated that this drug is closely associated with the nerve impulse throughout the central nervous system. His findings will be reviewed later in this study.

The Problem. This study was designed to discover the effects of local applications of acetylcholine to the cerebral cortex of the rat in conjunction with forced reaching on handedness.

Importance of the Study. A theory concerning the role of acetylcholine in neural impulses is propounded by Nachmansohn which states that this drug, when released along the nerve membrane, is responsible for the action potential of the nerve.⁸ He states the evidence that supports his claims that acetylcholine is found in quantities paralleling the intensity of neural activity, and that it plays the same role in the nerve impulse transmission along the axon as it does at the synapse. Peterson has suggested that acetylcholine may be the neuro-chemical equivalent of practice.⁹ If an experimental design of this nature would yield positive results it would support the theories outlined and suggest a new approach for

⁸ Nachmansohn, D., "The Role of Acetylcholine in the Mechanisms of Nerve Activity," Vitamins and Hormones, 1945, 3, 337-377.

⁹ Peterson, G. M., op. cit., p. 411.

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investigation.

Review of the Literature. During the last century the scientists interested in the mechanisms of nervous function were limited mainly to the study of electric changes during nerve activity in that other means to handle the problem were unavailable. With the better methods that were developed later the field of electrophysiology advanced rapidly, but an electrical analysis is not sufficient to understand the mechanism involved in nervous function. Nerve activity requires energy and in living organisms chemical reactions are the only conceivable source.

Several early scientists suggested that a chemical substance might intervene in transmission of impulses, but they had little evidence until Otto Loewi performed his experiment with frog hearts in 1921.¹⁰ He found that when the vagus of the frog's heart was stimulated there appeared in the Ringer's solution filling the heart a substance, which, if transferred to another heart, reproduced the effect of vagus stimulation. It was concluded that vagus stimulation results in the liberation of a chemical substance which affects the heart muscles

¹⁰ Loewi, O., Harvey Lectures, 28 (1932-33), 218 (1934), cited by Nachmansohn, op. cit., p. 338.

Investigation.

Review of the Literature

For the scientists interested in the nervous system, the functions of the nervous system were limited mainly to the study of the changes during nerve activity in the nervous system. In the past, the problem was investigated by the use of methods that were developed during the study of physiology advanced rapidly, but the methods employed are not sufficient to understand the nervous system in nervous function. Nerve activity is not only in living organisms, chemical reactions are not only conceivable as such.

Several early scientists suggested that a chemical

substance might intervene in the transmission of impulses,

but they had little evidence until the work of Sherrington

his experiment with frog nerves in 1897.

When the vials of the frog's nerve were placed in a solution

appeared in the Ringer's solution. This substance, which, it

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produced the effect of nerve impulses. It was found

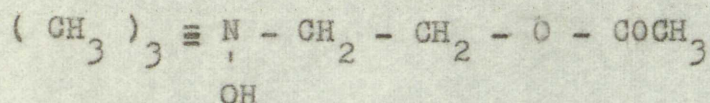
cluded that vague stimulation was not the same as the stimulation

of a chemical substance which affects the nervous system.

To Lewis, C. C. Harvey, and others, the substance was called (1924), called by Ransom, the "Ringer's solution".

directly. It was named "Vagusstoff" and was later identified as acetylcholine.

Acetylcholine is an ester formed from choline and acetic acid. Its structural formula is:



This ester is hygroscopic, very soluble in water and alcohol, and is easily decomposed by heat or alkali.¹¹ In living tissue acetylcholine is very unstable. It is hydrolyzed by the respiratory enzyme, cholinesterase, to form acetic acid and choline. Since cholinesterase is believed to be peculiar to this metabolic function alone, its abundance in nerve tissue is used as an indication of the amount of acetylcholine that is present. This indirect measurement is necessary because small units of nerve tissue do not yield enough acetylcholine to be measured accurately.¹² Nachmansohn and associates used this measuring technique to determine the amount of acetylcholine present in the electric organs of the eel.¹³ The *Electrophorus electricus* is particularly favorable material because the voltage and number of nerve end

¹¹ Goodman and Gilman, The Pharmacological Basis of Therapeutics. New York: The Macmillan Company, 1941, p. 351.

¹² Nachmansohn, D., op. cit., pp. 341-344.

¹³ Ibid., pp. 351-358.

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Acetylcholine is a colorless, odorless, and
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This ester is hydrolyzed very rapidly in water and
alcohol, and its activity disappears as fast as it is
In living tissue acetylcholine is very unstable and is
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is believed to be reutilized in the synthesis of acetylcholine
alone, its abundance in nerve tissue is much less than in the
distillation of the amount of acetylcholine that is present.
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11 Goodman and Gilman, The Pharmacological Basis of Therapeutics,
New York: The Macmillan Company, 1955, p. 351.

12 Nachmansohn, Ann. N.Y. Acad. Sci., 1954, 52, 1-14.

13 Ibid., pp. 354-355.

plates per centimeter decrease in an "S" shaped curve from head to tail end of the organism. They found that the concentrations of cholinesterase also decrease in an "S" shaped curve in the same manner as the plates and voltage. This parallelism is strong evidence that the acetylcholine metabolized by the cholinesterase is intrinsically connected with the electric charge in that no other enzymes or compounds tested revealed the same curve. Since these findings indicate that the enzyme, cholinesterase, is exclusively located along the entire neuronal surface it suggests that the ester, acetylcholine, is not limited to nerve endings, but is intimately connected with the total mechanism of impulse transmission.

Nachmansohn explains the nervous conduction to be a result of a change of permeability of the polarized membrane surrounding the nerve fiber. The change of the permeability permits the K ions to interchange and depolarization results. Since this point is now negative to the adjacent polarized region the current flows to the depolarized region and the action of depolarization continues along the fiber. Nachmansohn believes that it is the release of acetylcholine over this polarized membrane that causes the decreased resistance that permits depolarization. Polarization is then rapidly restored by the removal of the free ester

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by hydrolysis actuated by the enzyme, cholinesterase. In this process phosphate bonds would supply the energy for restoration of the resting condition of the nerve. The impulse is able to cross at the synapse because the increased surface lowers the resistance. The question still remains as to the nature of the mechanism that releases the acetylcholine.¹⁴

The theory propounded by Nachmansohn is based on an intracellular, in contrast to the older, extracellular concept of acetylcholine activity. His arguments are based on several facts: (1) The amount of acetylcholine found in perfusion fluids fall far short of the amount needed to have a stimulating effect. He accounts for the presence of any of the ester in the perfusion fluid to be due to leakage resulting from the techniques necessary to get at the event. (2) Cholinesterase is most highly concentrated inside the nerve fiber. This would be difficult to explain in an extracellular theory which states that the ester acts only after leaving the cell.¹⁵ (3) In an experiment conducted on the giant axons of the squid they found that acetylcholine did not penetrate the axonal surface membrane due to the lipoid material. The effect was evident only at the synapse where the

¹⁴ Ibid., pp. 358-360.

¹⁵ Ibid., pp. 368-371.

by hydrolysis catalyzed by the enzyme, cholinesterase. In this process phosphate bonds would supply the energy for restoration of the resting condition of the nerve. The impulse is able to cross at the synapse because the increased surface lowers the resistance. The question still remains as to the nature of the mechanism that releases the acetylcholine.

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¹⁴ Ibid., pp. 358-360.

¹⁵ Ibid., pp. 368-371.

lipoid material was absent which indicates that along the axon the ester must operate in an intracellular manner.¹⁶

Miller, Stravsky, and Wootton applied acetylcholine to areas of the motor cortex of cats and rabbits and recorded the effects with an electroencephalograph.¹⁷ They noted a reduction in slow wave amplitude and attributed this to the asynchronous firing of many neurons. Chatfield and Dempsey found that it was necessary to apply two and one-half to ten per cent concentrations of acetylcholine to cause any observable changes in the electrical cortical activity of cats if no anti-cholinesterase were applied.¹⁸

In the study by Peterson referred to previously an attempt was made to cause a shift in preferential handedness by applying acetylcholine to the non-preferred

¹⁶ Rothenberg, Sprinson, and Nachmansohn, "Site of Action of Acetylcholine," Journal of Neurophysiology, 1948, 11, 111-116.

¹⁷ Miller, Stravsky, and Wootton, "Effects of Eserine, Acetylcholine, and Atropine on the Electroencephalocorticogram," Journal of Neurophysiology, 1940, 3, 131-138.

¹⁸ Chatfield and Dempsey, American Journal of Physiology, 1941, 135, 633, cited by Felberg, W., "Present Views on the Mode of Action of Acetylcholine in the Central Nervous System," Physiological Review, 1945, 25, 598.

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motor cortex of the rat.¹⁹ Of 108 single-handed rats transfer occurred in two, and of forty-two ambidextrous rats positive transfer occurred in fifteen. He also reported that the contralateral forelimb exhibited clonic reactions a few minutes after the drug was applied. This seems to indicate that the drug reached the motor neurons innervating the limb. Peterson and Rigney continued this problem by using other chemicals in conjunction with acetylcholine.²⁰ The purpose of the additional chemicals was to stabilize the acetylcholine. Their results indicate that none of the combinations produced a marked increase in transfer of handedness, but some did permit a lesser concentration of acetylcholine to take effect.

Methods of Procedure. This experiment was designed to avoid the relative unreliability of ambidextrous rats. While working with ambidextrous rats Peterson studied the day to day reliability of reaches.²¹ In 101 instances of fifteen ambidextrous rats the average day to day change was thirteen per cent. It is also true that the lack of striking results while working with

¹⁹ Peterson, G. M., op. cit., pp. 404-412.

²⁰ Peterson, G. M., and Rigney, J. W., "Influence on Handedness of Acetylcholine Locally Applied with Other Chemicals to the Cerebral Cortex of the Rat," Journal of Comparative and Physiological Psychology, 1950, 43, 264-272.

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²¹ Peterson, G. W., op. cit., p. 407.

ambidextrous rats may be due to cortical injury causing negative results.

To avoid these obstacles single handed rats were selected, but previous work of this nature did not yield promising results.²² On the other hand, in a systematic study on the effects of forced reaching on handedness, Peterson found that 100 to 200 forced reaches changed handedness in about fifty per cent of his cases when original handedness was determined by only ten free reaches.²³

With these findings in mind the rationale of the present design is as follows: ostensibly single handed rats have developed a strong preferential handedness habit, so much so as to be unaffected by applications of acetylcholine. If forced reaching were introduced the rat would be prone to using either hand depending on the amount of forced reaching. With this greater degree of sensitivity in handedness the effects of acetylcholine may be more easily determined. If post-operational forced reaching were introduced those cases that are unable to reach due to a possible operational injury could be eliminated from the study, thus removing

²² Ibid., p. 406.

²³ Peterson, G. M., op. cit., "Transfers in Handedness in the Rat from Forced Practice," In Print.

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²³ Peterson, G. W., *et al.*, "Transfer in Handedness in the Rat from Forced Reasoning," in *Print*.

one more variable not pertinent to the study.

Single handed rats were selected by three periods of fifty free reaches. These rats were given 100 pre-operational forced reaches and fifty post-operational forced reaches followed by the crucial fifty free reaches. On operation day the control group had tap water applied and the experimental group had various concentrations of acetylcholine applied. Any significant difference found in the free reaches will be attributed to the drug versus the water.

The animals selected for the experiment ranged from three months to eight months of age. These ages were selected because the rats are mature enough to avoid stunting from starvation and are still active in the food situation. The two groups were equated to have similar ages, sex, and right and left handedness. Following is the day by day procedure for both groups.

Day one. Food was removed from the home cage.

Day two. The rats were placed into the free reaching food situation for orientation. In this situation a canary feeding dish extends into the cage. After the rats eat the food until the level is too low to be reached with their tongues they are forced to use either of their hands to obtain more. Since the food is as conveniently reached with either hand no bias is introduced.

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ing food situation for orientation. In this situation a canary feeding dish extends into the cage. After the rats eat the food until the level is too low to be reached with their tongues they are forced to manipulate of their hands to obtain more. Since the food is so conveniently reached with either hand no bias is introduced.

A reach is considered the act of grasping food and conveying it to the mouth. As soon as the rats started to reach for food on day two they were removed to their home cage for fifteen minutes of feeding.

Day three. The rats were placed into the free reaching situation and were permitted to take fifty reaches. Those rats taking ten or more reaches with the non-preferred hand were discarded from the study. They were returned to the home cage for fifteen minutes of feeding.

Day four. Repetition of day three.

Day five. The animals were given fifty free reaches and the entirely single handed rats were then placed into the forced reaching situation for a total of fifty forced reaches. This arrangement has a food dish adjacent to the wall of the cage with only limited room to make the reach. This condition makes it inconvenient to reach with the preferred hand so that the rat eventually uses the non-preferred hand to procure the food.

Day six. The rats were given fifty forced reaches in the offset food situation.

Day seven. The rats were anesthetized by ether. The operation was performed by making an incision through the scalp exposing the skull and trephining the frontal bone lateral to the superior sagittal suture and tangent to the coronal suture. A one-eighth inch trephine was

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used and the opening was made on the side contralateral to the non-preferred hand. Caution was exercised not to trepan too deeply so that the cortex and dura would remain intact. The control group had a small cotton bolus saturated with tap water placed over the hole in the skull. The experimental group had acetylcholine applied in place of water by the same method. The cotton was left in place and the incision in the scalp was closed with wound clamps. The rat was then placed into the forced reaching situation until fifty reaches were taken. The rat was then placed into the free reaching situation where fifty reaches were observed and handedness recorded.

Day eight and following. The rats were observed for fifty free reaches per day for six consecutive post-operational days. No further observations were necessary because all of the cases returned to original handedness and only insignificant variations were noted. After the follow-up observations each rat was sacrificed for an examination of the condition of the cortex. It was the intention to discard those rats with damage or infections from the operation, but there was not a single rat with cortical damage.

Results. A total of sixty rats were used in the study. Actually 126 rats were started, but sixty-six were discarded from the study for such reasons as refusing

used and the opening was made on the side contralateral to the non-preferred hand. Caution was exercised not to deepen too deeply so that the cortex and dura would remain intact. The control group had a small cotton ball saturated with tap water placed over the hole in the skull. The experimental group had castor oil applied in place of water by the same method. The cotton was left in place and the incision in the scalp was closed with wound clips. The rat was then placed into the forced reaching situation until fifty reaches were taken. The rat was then placed into the free reaching situation where fifty reaches were observed and handness recorded. Day eight and following. The rats were observed for fifty free reaches per day for six consecutive post-operational days. No further observations were necessary because all of the cases returned to original handness and only insignificant variations were noted. After the follow-up observations each rat was sacrificed for an examination of the condition of the cortex. It was the intention to discard those rats with damage or lesions from the operation, but there was not a single rat with cortical damage.

Results. A total of sixty rats were used in the study. Actually 120 rats were started, but sixty-six were discarded from the study for such reasons as retaining

to reach for food, death during the operation, or injury to the cortex during the operation.

Table I outlines the number of non-preferred reaches taken by each rat of both groups on operation day. The control group had a total of thirty-nine non-preferred reaches out of a possible 1500. (Fifty reaches per rat - thirty rats per group.) The experimental group had a total of 166 non-preferred reaches. The follow up observations were of the following nature. The experimental group took a total of thirty-eight non-preferred reaches on the day after the operation as compared to no non-preferred reaches for the control group. On the second post-operational day the experimental group took twelve as compared to seven non-preferred reaches of the control group. On the following observation days there were only slight variations because all of the rats had returned to original handedness.

It is also seen from Table I that the experimental group has thirteen cases where one or more reaches were taken with the non-preferred hand as compared to seven cases in the control group.

Of the four rats that had a twenty per cent concentration of acetylcholine all had clonic reactions of the contralateral forelimb after the operation. Three of the six rats that had a ten per cent concentration

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exhibited clonus, and of the remaining twenty rats with five per cent concentrations three had clonus. None of the control group had clonic reactions.

Table I has the rats in the order of the number of non-preferred reaches taken on operation day. In the experimental group those rats are designated with an asterisk that had concentrations of acetylcholine of more than five per cent. The other rats had five per cent concentrations.

Analysis. The distributions of non-preferred reaches taken on operation day for the two groups are plotted in the graph on page 18. It was originally hoped that the data would distribute themselves to be amenable to an analysis of variance, but the unusual characteristics of these distributions make this type of statistical analysis impossible. The distributions are severely skewed and therefore cannot be considered to be samples that were taken from a normally distributed population. An attempt was made to transform the data so that the analysis of variance would apply. The purpose of such a transformation is to change the scale of measurement to one in which the application of the analysis of variance is more likely to be valid, i.e., a scale in which the variance is more homogenous. The transformations may also have a desirable effect not only

exhibited almost, and of the remaining twenty rats with five per cent concentrations three had almost none of the control group had almost none.

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TABLE I
OPERATION DAY RECORDS OF REACHING

Control Group				Experimental Group			
Rats	NPR**	Rats	NPR	Rats	NPR	Rats	NPR
1.	29	16.	0	31*	48	46*	0
2.	3	17.	0	32.	25	47*	0
3.	2	18..	0	33*	24	48*	0
4.	2	19.	0	34.	16	49.	0
5.	1	20.	0	35.	14	50.	0
6.	1	21.	0	36.	12	51.	0
7.	1	22.	0	37*	11	52.	0
8.	0	23.	0	38.	5	53.	0
9.	0	24.	0	39*	3	54.	0
10.	0	25.	0	40*	3	55.	0
11.	0	26.	0	41.	2	56.	0
12.	0	27.	0	42.	2	57.	0
13.	0	28.	0	43.	1	58.	0
14.	0	29.	0	44*	0	59.	0
15.	0	30.	0	45*	0	60.	0
		Total	39			Total	166

*Rats with acetylcholine concentrations over five per cent.

** NPR - Number of non-preferred reaches in a total of fifty reaches.

TABLE I
OPERATION DAY RECORD OF REACHING

Control Group				Experimental Group			
Rate	NR*	Rate	NR	Rate	NR	Rate	NR
1.	29	15.	0	319	48	457	0
2.	3	17.	0	32.	25	494	0
3.	2	18.	0	337	24	487	0
4.	2	19.	0	34.	16	49.	0
5.	1	20.	0	35.	14	50.	0
6.	1	21.	0	36.	12	51.	0
7.	1	22.	0	377	11	52.	0
8.	0	23.	0	38.	5	53.	0
9.	0	24.	0	394	3	54.	0
10.	0	25.	0	407	2	55.	0
11.	0	26.	0	41.	2	56.	0
12.	0	27.	0	42.	2	57.	0
13.	0	28.	0	43.	1	58.	0
14.	0	29.	0	447	0	59.	0
15.	0	30.	0	457	0	60.	0
Total 39				Total 166			

*Rate with acetylcholine concentrations over five per cent.

**NR - Number of non-preferred reaches in a total of fifty reaches.

of stabilizing the variance, but also of decreasing the skewness or non-normality of the variable. Unfortunately none of the methods of transformation are effective on our particular data.

A second choice was to test the distributions for a Poisson "fit," but a Chi Square test of this assumption indicated that the data did not fit the Poisson distribution at the one per cent level of confidence.

By the use of a Chi Square test of independence using rats as frequencies the two groups differed at the ten per cent level of confidence. The Chi Square operations are on Table III. Little faith is given to this statistic for this particular type of data. As can be seen from Table III all of the rats taking any reaches are lumped into a single classification. This is not sensitive to those rats that have taken many reaches and it is believed that the low level of confidence results from this conservative statistic.

The control group took a total of thirty-nine reaches with the non-preferred hand and the experimental group took 166 making a total of 205 non-preferred reaches. Of this total the experimental group took eighty-one per cent in contrast to the control which took only nineteen per cent.

From the thirty rats in the control group, only

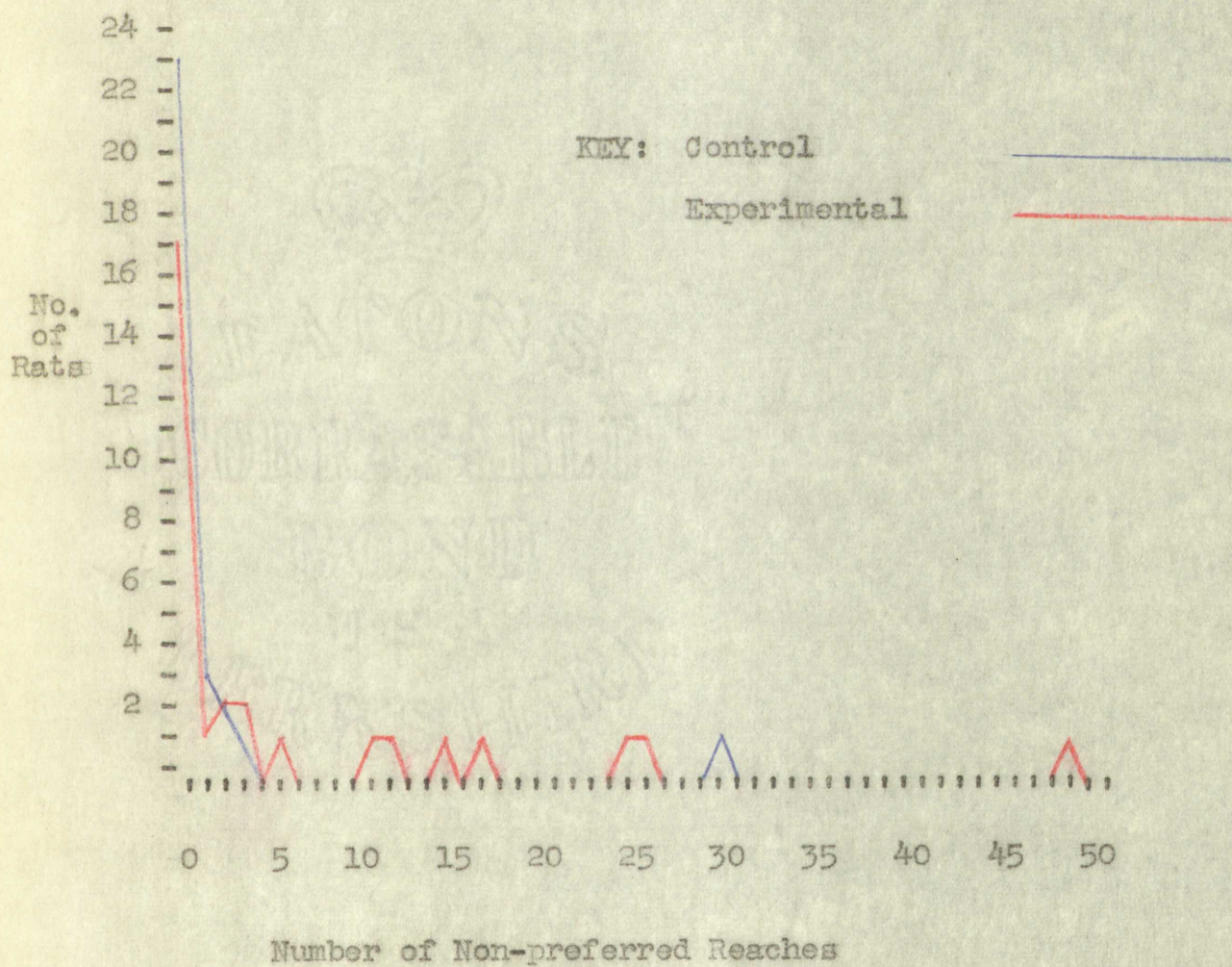
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From the thirty rates in the control group, only



seven took reaches with the non-preferred hand as compared to the experimental group which had thirteen rats take reaches with the non-preferred hand. Of the twenty rats that took non-preferred reaches sixty-five per cent came from the experimental group and thirty-five per cent from the control group. These percentages strongly indicate that acetylcholine was facilitating but no statement of confidence can be made.

Discussion. There is still a question to be raised concerning the results of this experiment. There are several possible answers why some rats were affected and others not. (1) We are only able to measure the actual reach, but there may have been an "urge" not sufficiently strong to overcome the habit of the preferred hand. If this were the case more positive transfer might have resulted if more forced reaching were introduced into the design. This would affect both groups, of course, but the experimental group might have made a more significant and striking transfer. (2) The relatively crude methods used in applying the drug to the cortex allows too much room for variability, i.e., there is no way of knowing how much of the drug, if any, is reaching the desired motor area. In some cases the drug may be absent from the area and in other cases it may be of sufficient strength to be damaging to the tissue

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TABLE II

CHI SQUARE OF INDEPENDENCE USING
FREQUENCY OF RATS

	NO*	YES*	TOTALS
CONTROL GROUP	(20)** 23	(10)** 7	30
EXP. GROUP	(20)** 17	(10)** 13	30
TOTALS	40	20	30

* No designates number of rats taking no reaches with the non-preferred hand.
Yes designates the number of rats taking from 1 to 50 reaches with the non-preferred hand.

** Independence values (expected frequency)

$$\left(\frac{40 \times 30}{60} \right)^2 = 20 \quad (fe)$$

$$\left(\frac{20 \times 30}{60} \right)^2 = 10 \quad (fe)$$

$$\text{Chi Square} = \frac{3^2}{20} + \frac{3^2}{20} + \frac{3^2}{10} + \frac{3^2}{10} = 2.7$$

At 1 df. 2.7 is significant at the 10% level.

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TOTALS	FREQUENCY OF RATS		TOTALS
	NO*	YES*	
30	23	7	30
	(20)**	(10)**	
30	17	13	30
	(20)**	(10)**	
TOTALS		40	60

* No designated number of rats taking no
resolves with the non-preferred hand.
Yes designated the number of rats taking
from 1 to 30 resolves with the non-pre-
ferred hand.

** Independence values (expected frequency)

$$= \frac{(40 \times 30)}{60} = 20 \quad (1e)$$

$$= \frac{(20 \times 30)}{60} = 10 \quad (1e)$$

$$\text{CHI Square} = \frac{23}{20} + \frac{7}{10} + \frac{17}{20} + \frac{13}{10} = 2.7$$

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and therefore inhibiting. These few cases that are greatly affected may be due to a proper and effective amount of acetylcholine, and if this could be controlled the results would be more convincing.

In a future study of this nature I would suggest that the rats would have their original handedness determined by only ten free reaches so that their preferential handedness habit will not be additionally reinforced. The forced reaches would then have a greater effect. According to Peterson's study referred to earlier 150 forced reaches would be sufficient to cause a fifty per cent transfer in the control group. This indicated that the rats are prone to using either hand and it is conceivable that if acetylcholine is facilitating the experimental group should be greatly affected.

Summary and Conclusions. Sixty rats were used in an experimental design to determine the effects of acetylcholine applied to the motor cortex and forced reaching on handedness. They were divided into two groups: the experimental, which had five, ten, and twenty per cent concentrations of acetylcholine applied to the non-preferred motor cortex; and the control, which had tap water applied in the same manner.

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two groups due to the unusual distribution of the data. A mere analysis of the raw data strongly indicates that acetylcholine is facilitating in causing transfer of handedness. A continuation of this study might reveal more significant results if the present design is altered as suggested.

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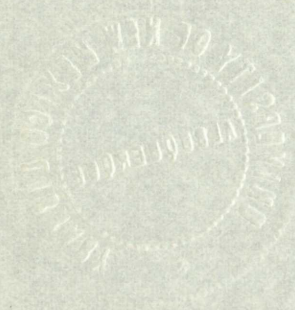
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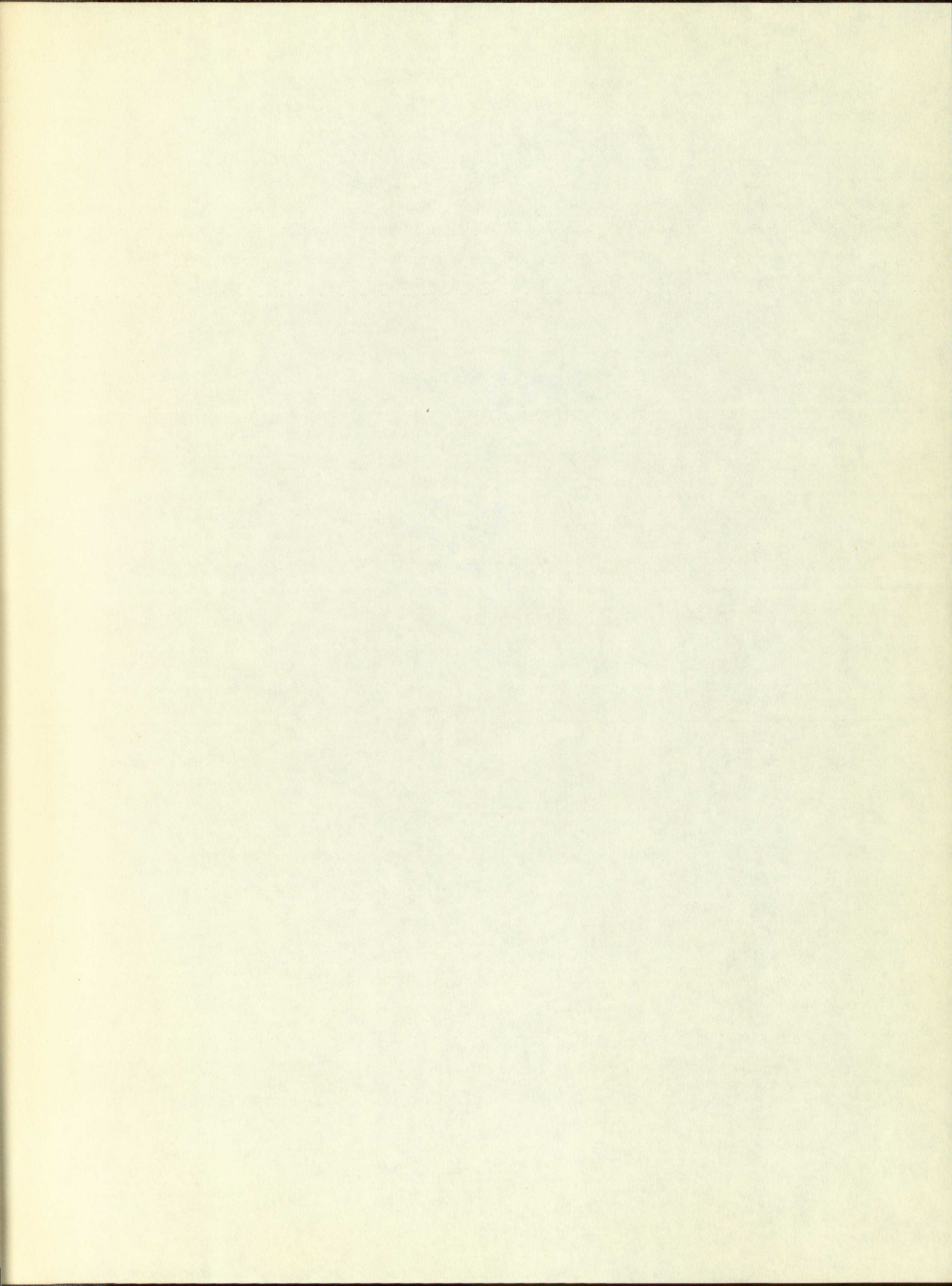


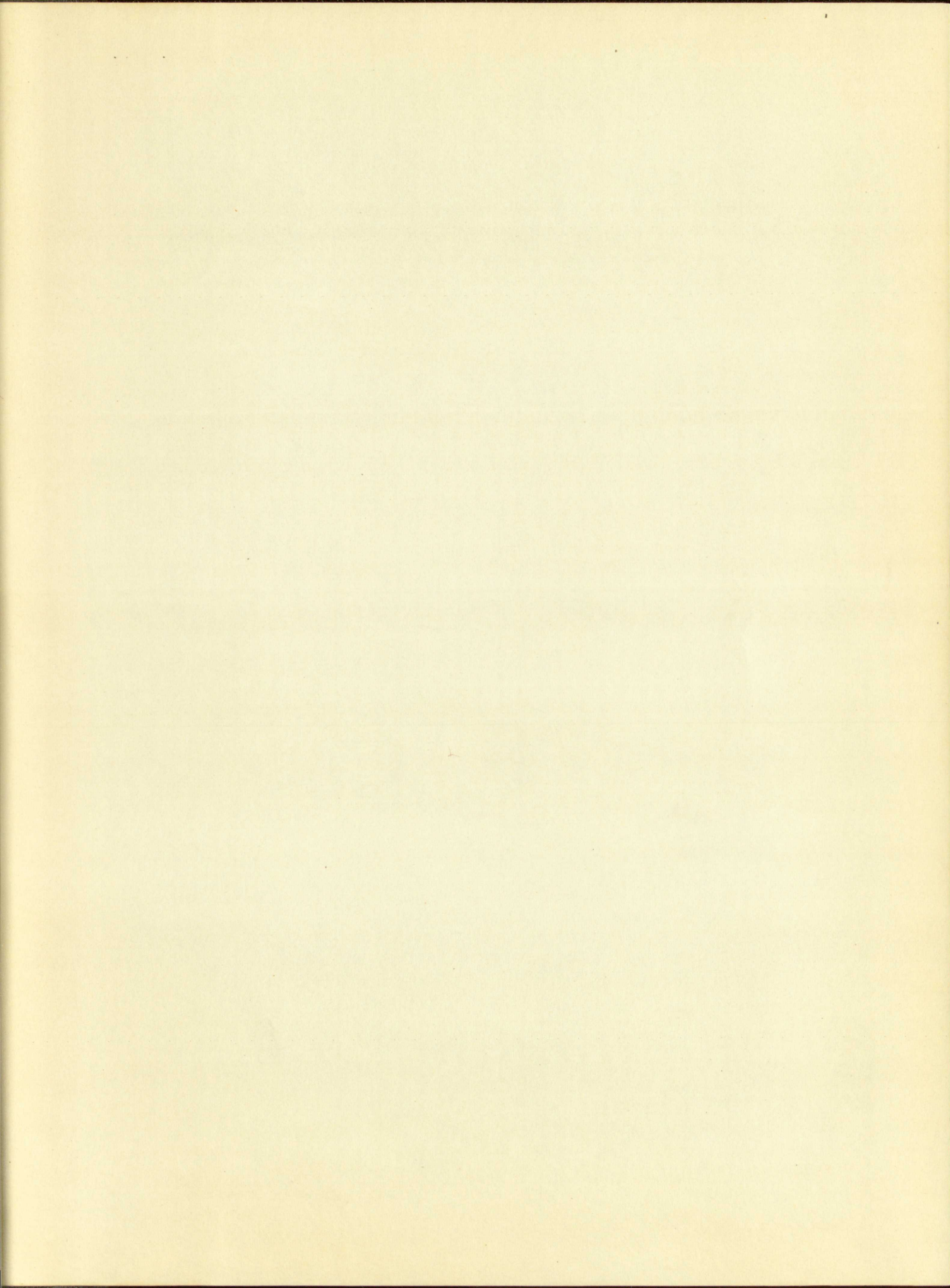
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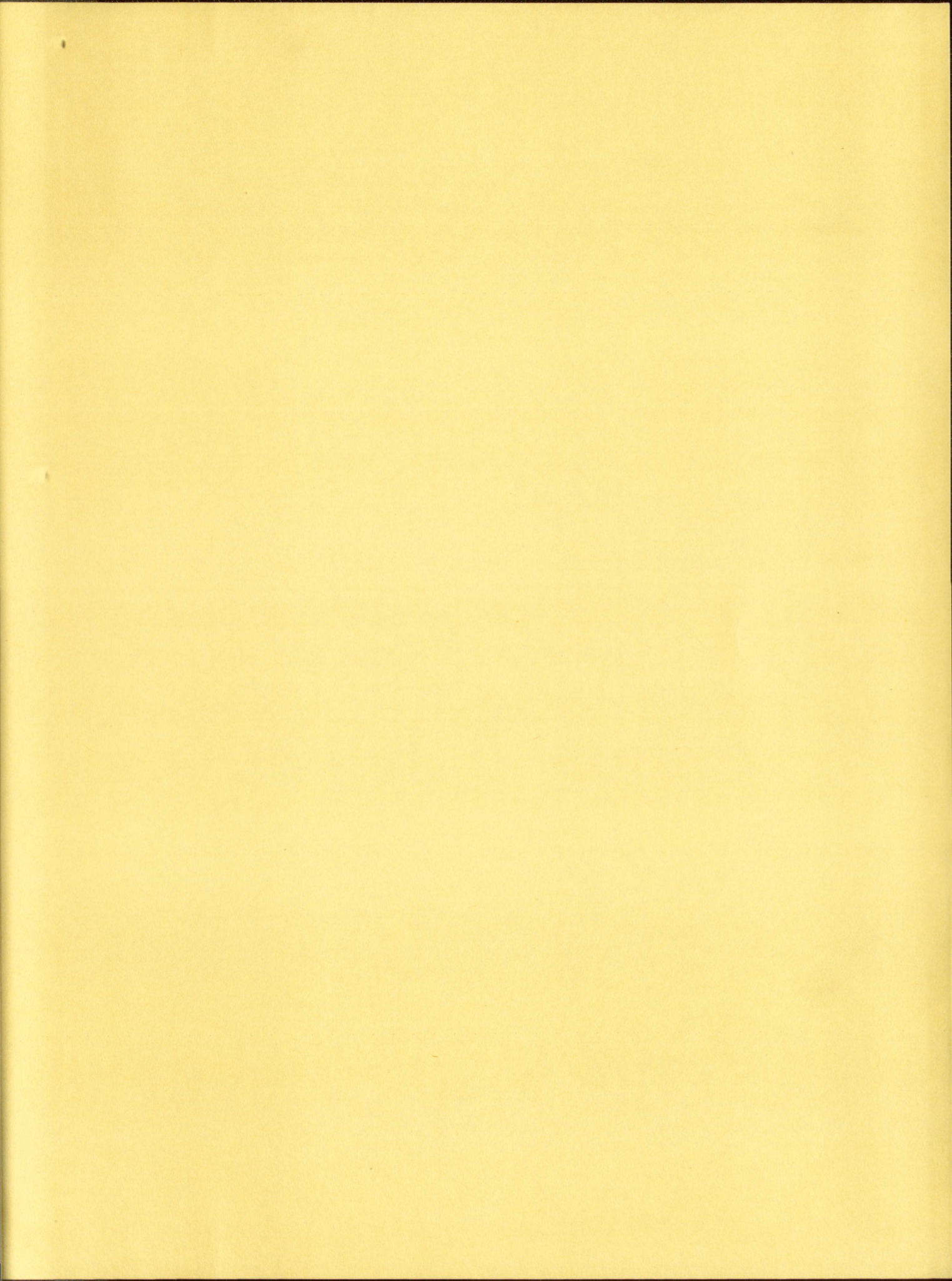
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